MEDICAL LABORATORY METHODS

H. FRENCH

SECOND EDITION

MEDICAL LABORATORY METHODS AND TESTS

BY

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PREFACE TO THE SECOND EDITION

Not only have many new methods and tests been included and the letterpress considerably altered from that of the first edition, but also most of the diagrams have been redrawn. It has been found necessary to use a heavier paper. Nevertheless, the original object of keeping the volume as small as possible has been maintained; and as a small handbook of the commoner laboratory methods used in medicine it is hoped that it will continue to be of service both to medical students and to practitioners.

HERBERT FRENCH.

Guy's Hospital,
November 11, 1907.

PREFACE TO THE FIRST EDITION

This volume has been written in response to repeated complaints that there was no *small* book dealing with the chemical and microscopical tests and investigations which are most useful to medical men. The object has been to detail the commoner methods, pointing out the conclusions which may be drawn from the various tests, but laying stress upon the fallacies to which each is liable. The book deals, not with the examination of patients, but with that of fluids or substances obtained from them, and bedside methods have been excluded. It is intended to be a small handbook for the medical laboratory.

HERBERT FRENCH.

Guy's Hospital,
March 19, 1904.

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CHAPTER I

EXAMINATION OF THE URINE

I. GENERAL CHARACTERISTICS OF URINE.

THE **amount** passed in twenty-four hours is, roughly, 1,500 c.c., or 50 ounces; but varies within wide limits. Free perspiration diminishes it, as in summer and after exercise; it is increased in winter and after copious drinking. As passed, it is usually quite clear. On standing, a faint cloud of 'mucus' often forms, especially in women. A dense pink precipitate of urates is common in summer urine; a white precipitate of earthy phosphates is not unusual in the 'alkaline tide' which follows a full meal. Some common pathological changes in amount are:

Increase in—		*Decrease in—*
Granular kidney, 18 to 100 ounces.		Fevers, such as pneumonia—*e.g.*, 25 ounces.
Diabetes mellitus, 100 to 200 ounces.		Acute nephritis—*e.g.*, 10 ounces, or even none at all.
Diabetes insipidus, 100 to 300 ounces.		Heart failure—*e.g.*, 20 ounces, 10 ounces, or less.
Hysteria, After epileptic fit, After head injury, Recovery from anasarca,	Up to 100 ounces.	After catheterization it may be entirely suppressed.

The **specific gravity** is determined by the urinometer, the scale being read at the point where the graduated stem leaves the fluid. If there be insufficient urine to float the urinometer, add an equal bulk of distilled water; multiply the last two figures of the specific gravity of the mixture by 2 to give that of the original urine. In health it varies from 1002 after much drinking to 1030 after profuse perspiration.

Pathological Changes in Specific Gravity:

Unduly low—	*Unduly high—*
Diabetes insipidus, 1002 or 1004.	Heart failure, 1030 to 1035.
Hysteria, 1004.	Acute fevers, 1030 to 1035.
Post-epileptic, 1004.	Diabetes mellitus, 1035 to 1045.
Granular kidney, 1008 to 1012.	

The **reaction** is determined with litmus paper, 'acid' urine turning it red, 'alkaline' blue; when both red and blue litmus papers are turned purple the reaction is termed 'amphoteric.' The acidity is due to acid sodium phosphate; an alkaline tide follows the big meal of the day, owing to the increased acid secretion in the stomach. The urine is more acid in those who eat meat; in vegetarians it may be continuously alkaline. On standing, the acidity at first increases, then diminishes, and is changed to alkalinity by conversion of urea into ammonia.

Pathological Changes in Reaction:

Increased Acidity.	*Alkalinity.*
In acute fevers—*e.g.*, pneumonia, acute rheumatism.	Suppuration in the bladder.
In the uric acid diathesis.	

The **colour** of normal urine, due to urochrome,

varies from pale straw-yellow (low specific gravity) to dark sherry (high specific gravity).

Pathological Changes in Colour (for tests, see p. 24 *et seq.*):

Pallor: in diabetes mellitus, diabetes insipidus, granular kidney, hysteria, epilepsy, head injury, excessive drinking.

Blood: may make the urine bright red, dark red, port-wine colour; brown, brown-black, or black.

Red-brown: after rhubarb, senna, chrysophanic acid.

Greenish yellow to greenish black: bile pigments; phenyl compounds, such as carbolic acid, salol, resorcin, guaiacol, creosote, alkapton, naphthaline, indican; melanin.

Bright yellow: after santonin.

Dichroic: urobilin, red by transmitted, green by reflected light.

Blue: after the administration of methylene blue.

The **smell** of normal urine is 'aromatic,' but becomes ammoniacal on standing.

Pathological Changes in Smell:

Ammoniacal: in cystitis.

Fæcal: from contamination with *Bacillus coli communis.*

Like violets: after the administration of turpentine.

Sweet: in diabetes mellitus.

After eating asparagus the urine has a very characteristic odour.

II. URINE DEPOSITS.

It is often possible to obtain sufficient deposit for examination by merely allowing the urine to stand in a conical specimen-glass for some hours, and then pipetting off that which has subsided to the bottom. It is much better, however, to use a centrifugalizing machine to further concentrate the deposit, and to ensure that the lighter particles—renal tube-casts, blood-corpuscles, and so forth—are really precipitated. A hand or other centrifugalizing machine is essential to every doctor who wishes to make thorough urinary examinations himself.

The **colour** of the deposit may help one to form some idea of its main constituent—for example:

A *white deposit* may be due to phosphates, oxalates, pus, mucus; in some cases to starch grains which have fallen into the urine after the use of a dusting-powder; or, rarely, to urates which have come down in a urine in which there was no uroerythrin to colour them pink.

A *pink deposit* is nearly always due to urates, which, though colourless themselves, usually carry down with them the urinary pigment uroerythrin.

A *dingy brown deposit* is very suggestive of phosphates that have carried down with them blood pigment, and therefore points to hæmaturia.

A *brick-red deposit* like cayenne pepper is due to uric acid.

A *dark crimson deposit* is due to blood.

It is clear, however, that there may be many different constituents in the same deposit, and that

more than a naked-eye examination is required. Microscopical investigation is essential.

The Method of examining a Centrifugalized Deposit from a Urine microscopically. — One of the best ways of examining a urinary deposit microscopically is as follows: Prepare a stock bottle of normal saline solution (0·6 per cent. of common salt in water), to which enough methylene blue has been added to make the colour deep; have cover-glasses, pipettes, and slides all ready cleaned, and some 2-inch squares of blotting-paper; pour off all the supernatant fluid from the tube containing the centrifugalized urine, leaving behind the deposit and as little urine as possible; transfer a single drop of the deposit to a microscope slide by means of a pipette; with a clean glass rod transfer 1 drop of the normal saline methylene blue solution to the same slide, putting it near, but not in, the drop of centrifugalized deposit. The glass rod can be kept in the stock bottle of normal saline methylene blue for the purpose, and it is most important not to get it contaminated by anything on the slide, or else tube-casts and so on may be transferred back to the stock bottle. Now take a cover-glass, and with its edge gently, but thoroughly, mix together the drop of urine deposit with that of methylene blue; then put on the cover-glass; remove the surplus fluid by laying a square of blotting-paper firmly over the top of it. The preparation is then ready for examination under both the low-power and the $\frac{1}{6}$-inch objective. The blue stains the cellular elements present, bringing out nuclei, and at the same time forming a good background in which

renal tube-casts, red blood discs, and crystals can be seen. It is most important when examining with the microscope to shut down the diaphragm as much as possible; many things will then be visible which would escape detection if the full light were employed.

Although many different constituents will be found in the same deposit, it is more convenient to describe each separately; and for this purpose it is of service to divide them into three groups, namely:

A. Crystalline deposits.
B. Amorphous non-organized deposits.
C. Organized deposits.

A. CRYSTALLINE DEPOSITS

1. **Calcium Oxalate.**—This forms colourless crystals, really octahedra (Fig. 2), but looking like squares with a diagonal cross — so-called 'envelope' crystals (Fig. 1). They vary greatly in size, some being only just visible under the $\frac{1}{6}$-inch objective, others easily seen with the low power.

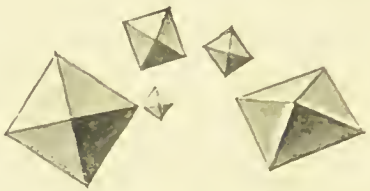

FIG. 1.—'Envelope' Crystals of Calcium Oxalate.

FIG. 2.—Diagram to show Octahedral Character of a Calcium Oxalate Crystal.

Importance.—They occur in many healthy acid urines, and are particularly abundant after eating rhubarb, tomatoes, cauliflower, and some other vegetables. They are therefore by no means always of pathological significance.

When so persistent as to constitute 'oxaluria,' they are a sign of acid dyspepsia.

The presence of many calcium oxalate crystals leads to irritation of the urinary passages, with the result that the urine often contains excess of nucleo-proteid at the same time (see p. 43), and, in the male, even considerable numbers of spermatozoa (see p. 19).

When the other clinical signs suggest the presence of a renal or vesical stone, the occurrence of abundant envelope crystals, together with red corpuscles, affords strong confirmatory evidence in favour of a 'mulberry' calculus.

2. **Uric Acid.**—The crystals are always coloured yellowish brown by the urochrome, and have a variety of shapes, the commoner being 'whetstones' and 'rosettes' (Fig. 3). 'Dumb-bells' and 'bundles' are rarer forms, due to incomplete crystallization. The crystals occur in acid urine as a deposit which looks to the naked eye like grains of cayenne pepper.

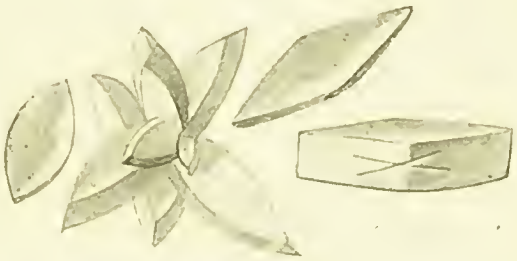

FIG. 3.—Uric Acid Crystals.
(Whetstone and Rosette Forms.)

Importance.—They indicate hyperacidity of the urine. The condition is not necessarily gouty, nor is the uric acid necessarily in excess. The process by which the uric acid becomes precipitated is, briefly, as follows: The NaH_2PO_4, to which the acidity of the urine is due, tends to remove Na from combination with uric acid, forming Na_2HPO_4, and converting the soluble

sodium quadriurate first into the less soluble sodium biurate, then removing the sodium even from the latter, so that the comparatively insoluble uric acid goes out of solution. It is only when the NaH_2PO_4 is in considerable excess—for example, upon a diet rich in meat—that the uric acid is precipitated to any extent. The hyperacidity may cause irritability of the bladder or urethritis, and the crystals may form a calculus. Treatment should be directed to diminishing the acidity of the urine in order to keep the uric acid in solution.

3. **Ammonio-Magnesium Phosphate** ($NH_4.Mg.PO_4$)—described as 'coffin-lid' or 'knife-rester' crystals (Fig. 4); colourless.*

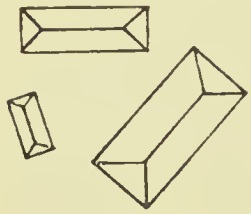

FIG. 4.—Ammonio-Magnesium Phosphate Crystals.

Importance. — They occur in urines which contain ammonia. To the naked eye the deposit is heavy, opaque white, and may be mistaken for pus. The ammoniacal change may have occurred since the urine was passed; the crystals then have no pathological import. If found in fresh urine, they indicate cystitis, or other cause of decomposition within the urinary tract.

4. **Earthy Phosphates**:

(*a*) **Calcium Phosphate.**—This is usually amorphous; rarely it assumes the form of colourless elongated prisms, occurring singly, or in starlike clusters of 'stellar phosphate' (Fig. 5).

* The term 'triple' was formerly used for this variety of phosphate, but it is incorrect.

(*b*) **Magnesium Phosphate.**—This is also amorphous as a rule, but occasionally it occurs in the form of large colourless prisms, not very unlike those of ammonio-magnesium phosphate. The points of distinction will be seen on comparing Figs. 4 and 6.

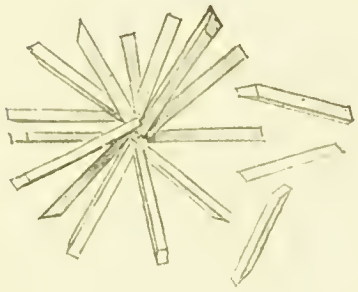

FIG. 5.—Stellar Phosphate Crystals.

Importance.—'Earthy,' as distinct from alkaline or ammonio-magnesic, phosphates do not indicate disease. They occur in faintly acid, neutral, or alkaline urines. They are abundant in the urines of vegetarians, or in the alkaline urine which is passed soon after a very big meal. After a big dinner, for example, the first urine voided may be milky-looking, owing to precipitation of earthy phosphates.

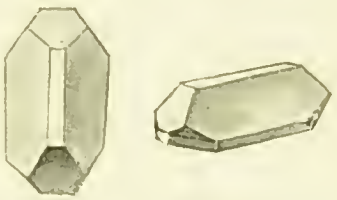

FIG. 6.—Magnesium Phosphate Crystals.

FIG. 7.—'Thorn-apple Crystals.'

5. **Sodium Urate and Ammonium Urate.**—Imperfectly formed 'thorn-apple' crystals, seen under the high power as small spheres with numerous short spines (Fig. 7).

Importance.—Both forms are distinctly rare. Sodium urate crystals have been noted in the urine of newly-born infants. 'Thorn-apple' crystals in adults are due to ammonium urate, in alkaline urines, in association with ammonio-magnesic phos-

phate. They indicate decomposition, either after the urine has been passed, or, in cases of cystitis, within the urinary tract.

6. **Tyrosine.**—Sheaves of colourless needles (Fig. 8), to be distinguished from phenyl-glucosazone crystals by (1) their smaller size, (2) their being colourless.

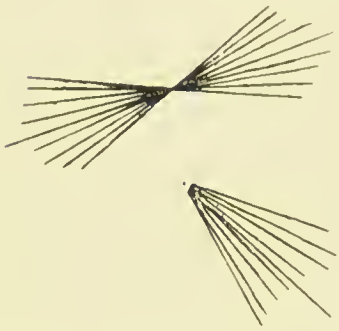

FIG. 8.—Tyrosine Crystals.

Importance. — They are rare; are usually associated with leucin; and are most typically seen in cases of acute yellow atrophy of the liver. They may be found in other affections of the liver also—*e.g.*, in phosphorus poisoning, and occasionally in pernicious anæmia.

7. **Leucin.**—Pale yellow spherical masses, often concentrically striated (Fig. 9).

Importance.—The same as that of tyrosine. Leucin and tyrosine are amido-compounds which, in health, should be converted into urea by the liver. Starch grains may be mistaken for leucin. (See p. 13.)

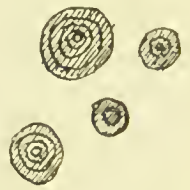

FIG. 9.—Leucin Spherules.

8. **Cystin.**—Colourless hexagonal plates in acid urine (Fig. 10).

Importance.—Cystin is a rare constituent of urine; but, if present in one member of a family, it is liable to occur in others also. It does not necessarily indicate ill-health, but there are great peculiarities in the metabolic processes of cystinuric

subjects. The condition is of too great rarity to warrant further discussion here.

Cystin has given rise to calculi which have a peculiar greenish or yellowish hue and soapy feel.

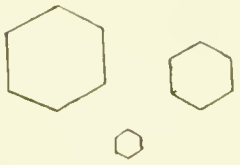

FIG. 10.—Cystin Crystals.

B. AMORPHOUS NON-ORGANIZED DEPOSITS

1. **Urates.**—Sodium and potassium urates are often found precipitated in concentrated acid urines, which, though clear when passed at body temperature, have become clouded on getting cold. Their precipitation depends upon the inability of the urine to keep as many urates in solution when cold as when hot. They have great affinity for uroerythrin, which gives the deposit a characteristic pink colour. In the absence of uroerythrin urate deposits are white like phosphates. Under the microscope, granular particles are seen running together into moss-like clumps.

To confirm, warm the urine to body temperature; urates redissolve. In case albumin be present, fill 3 inches of a test-tube with the urine and its deposit; warm the upper 2 inches gently. Urates will clear up. Boil the top inch; albumin, if present, will form a cloud, so that in the top inch there is a cloud of albumin; in the middle inch clear urine in which urates have redissolved at body-heat; at the bottom the original deposit of urates.

Importance of Urates.—They indicate a concentrated urine, but afford no clue to the cause of concentra-

tion. It may be physiological—for instance, in the summer-time, or after violent exercise or a Turkish bath, when the quantity of sweat lost by the skin causes the amount of urine to diminish; or it may be due to pathological conditions which diminish the total quantity of urine passed, such as acute fevers, heart failure, or excessive loss of fluid by vomiting or diarrhœa.

2. **Earthy Phosphates**—*i.e.*, *Phosphates of Calcium and Magnesium.*—To the naked eye the deposit is white and more or less dense and opaque. It may be confused with thick mucus, pus, alkaline phosphates, or colourless urates. When blood is present, the methæmoglobin tinges the phosphates a dirty brown. The urine may be alkaline, amphoteric, or just acid. Under the high power granular particles not unlike those of amorphous urates are seen, with an occasional crystal of stellar (calcium) or magnesium phosphate.

To some of the deposit in a test-tube add acetic acid; phosphates will redissolve; pus or urates will remain unaltered; mucus will become more marked. It is essential that acetic, and not nitric, acid should be used in performing the test; for nitric acid, though it would redissolve the phosphates, would at the same time precipitate any albumin present.

Phosphates, being less soluble in hot than in cold urines, often form a cloud on boiling. This clears up at once on the addition of acetic acid.

Importance of Earthy Phosphate Deposit.—It has no pathological significance; it indicates that the urine is not very acid.

3. **Starch Grains.**—These appear under the micro-

scope as round or oval bodies, with crescentic markings. They may be detected by their blue colour on adding iodine.

Importance.—They are adventitious, from dusting-powders, but may be mistaken for leucin until the iodine test has been applied.

C. ORGANIZED SEDIMENTS

1. **Epithelium**:

(*a*) **Squamous Cells.**—These are of large size, with a small central nucleus. They occur singly (Fig. 11), or in groups of two or three, or in sheets of twenty or more.

FIG. 11.—Squamous Cells.

Importance.—They are derived from the vagina. If very numerous, they indicate vaginitis and catarrh; in smaller numbers they have no significance.

(*b*) **Bladder Cells.**—Though variable in appearance, these are of two types. Those from the deep layer in the bladder are oval at one end, and at the other taper into a long or short tail (Fig. 12, B); the superficial cells are convex on thei free surface, with concavities on their under side, into which the oval cells of the deep layer have fitted (Fig. 12, A).

A B

FIG. 12.—Bladder Cells.
A, superficial; B, deep.

Importance.—An occasional surface cell has no particular significance. If present in numbers, vesica

catarrh is probable. When pyriform cells from the deeper layer are found, there is probably ulceration of the bladder.

(*c*) **Renal Cells.**—It is by no means always possible to say for certain from their microscopical characters what is the source of each and every epithelial cell in the urine. Degenerate leucocytes and degenerate renal cells look much alike. When the epithelium is comparatively fresh, however, two kinds of renal cells may be recognized with some certainty, namely—

(i.) Cells from the walls of the renal tubules.

(ii.) Cells from the renal pelvis.

(i.) **Cells from the Renal Tubules.**—These are about twice the size of leucocytes, and, when fresh, have a polygonal outline and central nucleus (Fig. 13, A). More frequently they have undergone fatty degeneration, and appear irregular, and opaque with fine granules, amongst which the nucleus is no longer distinguishable (Fig. 13, B).

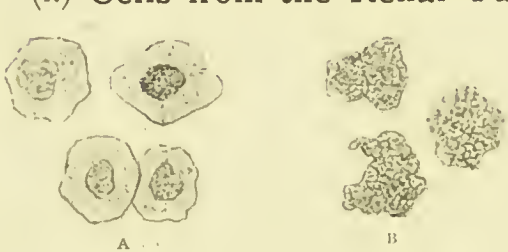

FIG. 13.—Renal Tubule Cells.
A, fresh ; B, fatty.

Importance.—They indicate a catarrhal condition of the kidney. They are particularly numerous in acute Bright's disease, but occur also in consecutive nephritis ; renal congestion from heart failure ; after infarction ; with renal calculus, tuberculous kidney, and with growth.

(ii.) **Cells from the Renal Pelvis.**—Cells from the renal pelvis have various shapes and sizes. Some are

ovoid and of comparatively large size ; some are polygonal, resembling those from the renal tubules. But the most characteristic renal pelvic cell is long and narrow, with a single nucleus and a slender, wavy tail (Fig. 14). It might at first sight be mistaken for a pyriform cell from the deeper layers of the bladder mucosa, but the tail is much longer and more tapering.

FIG. 14.—Tailed Cells from Renal Pelvis.

Importance.—The presence of these tailed cells indicates excoriation of the renal pelvis, such as is caused by pyelitis, calculus, or a tuberculous lesion. The cells are always pathological.

(*d*) **Fragments of New Growth.**—It occasionally happens that fragments detached from a new growth in the kidney or bladder are found in the centrifugalized deposit. The cells stain well with the methylene blue, and form continuous masses or clumps, sometimes with papillomatous projections. In the case of a bladder growth the particles are often large enough to be visible to the naked eye in the fresh urine ; fragments of renal growth are usually much smaller. The variety of cell seen will depend upon whether the growth is a papilloma, a sarcoma, or a carcinoma. One word of warning is needed : it sometimes happens that leucocytes or other cells clump together in the centrifugalized deposit in such a way as to resemble fragments of tissue. It is only by familiarity with the microscopical characters of urinary deposits that such artefacts can be distinguished from new growth.

Importance.—The finding of a fragment of new growth clinches the diagnosis. It must be remembered, however, that such fragments are not being constantly broken off from the main growth, so that in a suspicious case repeated examinations should be made if no fragment of new growth is found at first.

2. **Leucocytes.**—If fresh, these appear exactly like those of the blood, and are mostly polymorphonucleated; if the urine has stood, they become granular and fatty, but are distinguished from fatty renal cells by their smaller size (Fig. 15). Acetic acid renders the nucleus more distinct.

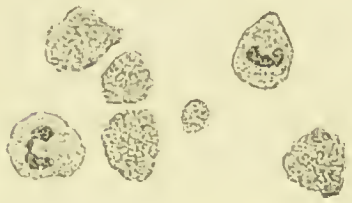

FIG. 15.—Pus Cells, Fresh and Degenerate.

Importance.—Small numbers indicate irritation within the urinary tract. They occur in Bright's disease, urethritis, vesical catarrh, and in association with blood in the urine. Large numbers indicate purulent inflammation, such as gonorrhœal urethritis, gleet, prostatitis, cystitis, renal growth, tubercle or calculus, or suppurative nephritis.

3. **Red Blood-Corpuscles.**—These often look like those of fresh blood; in very dilute urines they may be laked, and appear as 'ghosts'; in concentrated urines they may be crenated, and variously crumpled.

Importance.—In a woman they may be derived from menstrual blood. Otherwise, their presence is the best test of hæmaturia. They may be derived from the kidney, the ureter, the bladder, the prostate, the urethra, a caruncle, and the microscope affords no

evidence of their source. Apart from menstruation, they are always pathological.

4. **Renal Tube-Casts.**—There are the following varieties (Fig. 16):

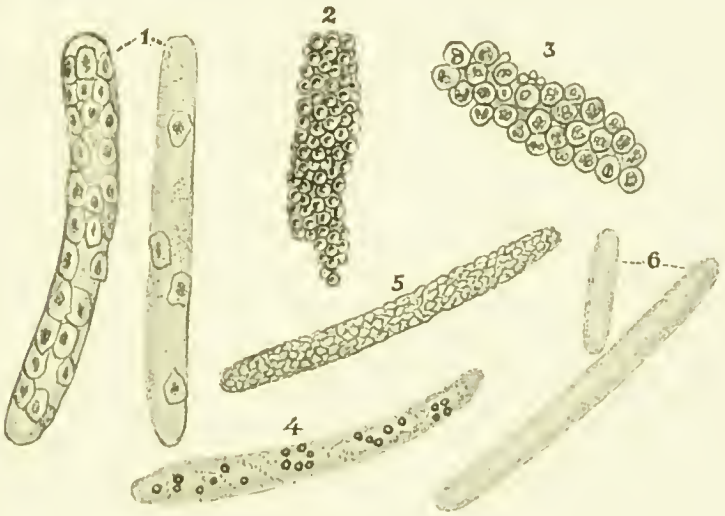

FIG. 16.—Renal Tube-Casts.

(1) Epithelial casts, in which outlines of renal cells are seen.

(2) Blood casts, composed of red corpuscles.

(3) Leucocyte casts, consisting of white corpuscles, which are distinguished from renal cells by their smaller size.

(4) Fatty casts, not very different to granular casts, but containing here and there small, highly refractile specks and globules of fat.

(5) Granular casts, having no cell elements, and a typical granular appearance.

(6) Hyaline and waxy casts, seen only in outline, the cast itself having no internal structure.

Many become broken and unrecognizable; some appear of different structure in different parts—*e.g.*, granular at one end, epithelial at the other.

Importance.—It would seem probable that, whereas blood and leucocyte casts depend on exudation from vessels, epithelial, fatty, and granular casts are progressive downward changes in casts derived from the renal tubule cells. The precise nature of the hyaline material of a hyaline cast is not known. It is the result of inflammatory changes, and forms the matrix in which are embedded the epithelial cells, fat globules, or granular debris of the other forms of casts. A simple hyaline cast is one consisting of matrix only, with nothing embedded in it. An occasional granular or hyaline cast is to be found in healthy urine that has been much centrifugalized. Small numbers may be found when the kidney is passively congested, from failure of the right side of the heart. Blood casts may occur in blood diseases, and with other causes of hæmorrhage within the kidney substance, such as infarction, or venous thrombosis; but the presence of many casts points strongly to nephritis. No case of Bright's disease has been fully diagnosed until renal tube-casts have been found in the urine. The more acute the nephritis, the more rapidly will the casts be formed, and the less change will they have undergone; the vascular changes will be the more marked also, with exudation of red and white corpuscles. Therefore, epithelial, blood, and leucocyte casts indicate acute renal inflammation; as the latter subsides, blood and leucocyte casts disappear, epithelial casts grow fewer, and the fatty casts

increase; in chronic nephritis hardly any epithelial casts are seen, fatty casts are few, granular and hyaline preponderate.

Waxy casts are not essentially different to hyaline; they are more refractile and more easily seen, but have no significance that hyaline casts have not.

5. **Spermatozoa.**—These are readily recognized in urine. Under a high power each is seen to have an ovoid head about two-thirds the size of a red corpuscle, and a thread-like tail, usually broken off short, but, if complete, about as long as eight red corpuscles (Fig. 17).

FIG. 17.—Spermatozoa.

Importance.—Spermatozoa may be found in the urines of perfectly healthy adult males, but they should not be present continuously and in numbers. In themselves they are not pathological, but they may point to the existence of some irritating constituent in the urine. The commonest such irritant is oxalate of lime. If many envelope crystals are present, it is not at all uncommon, in the male, to find at the same time excess of mucus, excess of nucleo-proteid (see p. 43), and many spermatozoa. Treatment of the oxaluria, by diet or otherwise, readily cures the condition.

6. **Bacteria.**—Normal urine which is left exposed to the air in warm weather soon swarms with the *Micrococcus ureæ*, which converts urea into ammonium carbonate. Associated with it may be hosts of non-pathogenic bacilli.

Importance.—None, except that if not recognized they may be mistaken for organisms which cause

disease. If invasion by adventitious organisms is to be inhibited, some antiseptic such as boric acid or thymol should be added to the specimen, the amount so added varying with the volume of urine which it is desired to keep sweet. It is noteworthy that some urines decompose far less readily than do others. Those from patients with uncomplicated phthisis may resist decomposition for days.

The pathogenic organisms which may be found are—

Staphylococci.	Tubercle bacilli.
Streptococci.	Bacillus coli communis.
Gonococci.	Typhoid bacilli.

In examining for any of these, make a film of the centrifugalized deposit (p. 108). If it is particularly desired that none of the film should wash off in the process of staining, it is well to albuminize the slides or cover-slips as follows: Mix some fresh white of egg with nine times its bulk of water and filter it; smear the filtered egg-white upon one surface of the cover-slip, and allow it to dry unexposed to dust. Slips albuminized in this way can be kept for future use. To find out which surface is albuminized, breathe on it; the unalbuminized surface becomes opaque from condensed moisture, the other remains transparent. Smear the urine deposit thickly on this surface; allow it to dry; fix the film by passing it through a Bunsen flame five times.

For *tubercle bacilli* stain by the Ziehl-Neelsen method (p. 101).

Importance.—If present, they indicate a tuberculous lesion of the kidney, ureter, or bladder. If absent, a tuberculous lesion is not excluded. With care,

tubercle bacilli are found at the first examination in something like half the cases of tuberculosis of the kidney or bladder. In suspicious cases in which the first examination proves negative, the procedure should be repeated upon successive days. There is nearly always a good deal of pus in the urine when tubercle bacilli are present, but it is worthy of note that the bacilli have many times been found when there is no pus. Absence of pus, therefore, is not an absolute contra-indication to examination for tubercle bacilli.

A source of fallacy is the **smegma bacillus,** which also stains by the Ziehl-Neelsen method. It will not retain the stain, however, if the film be first immersed for ten minutes in warm caustic soda solution containing 5 per cent. of alcohol, whereas tubercle bacilli will still stain as before. Those who are familiar with urinary examinations can distinguish smegma bacilli from tubercle bacilli morphologically, the former being longer and better defined than the latter. The term 'smegma' might suggest that the smegma bacillus was confined to the male. This is by no means the case. It occurs not infrequently in the normal urine of the female as well as in that of the male, and may be present in a catheter specimen. It is, therefore, a very real source of fallacy in examinations for tubercle bacilli in urine. Another method of avoiding the fallacy is to immerse the films, just before counterstaining in carbol methylene blue, in a solution of 95 per cent. spirit containing 5 per cent. of strong hydrochloric acid. If left in this for five minutes before counterstaining, the red colour

will be discharged from the smegma, and not from the tubercle, bacilli.

For *gonococcus*, *Bacillus coli communis*, *typhoid bacillus*, *streptococcus*, and *staphylococcus*, stain with carbol methylene blue or carbol fuchsin (p. 108). Staphylococci and streptococci may also be stained by Gram's method (p. 108), the others not.

Importance.—All these organisms are pathogenic, and any of them may cause cystitis. Gonococci more often come from the urethra. Typhoid bacillus is sometimes excreted in what appears to be healthy urine long after convalescence from enteric fever, but cannot be distinguished by the microscope from *Bacillus coli communis*. If thorough identification of the various bacteria is required, extensive cultural methods are essential. These are beyond the scope of a student's or practitioner's clinical laboratory.

7. **Yeast Fungi** (Fig. 72, p. 129) have no pathological significance. In warm weather they commonly occur, particularly in diabetic urine which has stood open to the air.

8. **Fat Globules.**—Highly refractile particles or globules, soluble in ether, readily stained black by osmic acid, or red by Sudan III. (p. 92).

Importance.—In temperate climates it is very rare to find fat in the urine unless by accidental or intentional admixture of milk or oil—*e.g.*, after vomiting, or the passage of an oiled catheter. True chyluria results most commonly from infection with filaria in the tropics (p. 90).

9. **Bilharzia Hæmatobia.**—This is the only parasite likely to affect the urine. The mature worm occupies

the pelvic vessels. The eggs work their way through the walls of the latter, and reach the urine through the bladder wall, producing hæmaturia. Each worm is $\frac{1}{200}$ inch long and half as broad. With the low power it resembles a melon seed, with a short spine at one end, or, in some cases, on one side (Fig. 18). The contained embryo will not hatch in urine, but if as much of the urine as possible is poured off from the centrifugalized deposit, some clean, tepid water poured on to the latter, and the tube again centrifugalized, a drop of the deposit containing the ova in the tepid water may be watched under the microscope, and the embryos, if living, may be observed bursting their chitinous shells and then swimming about free in the water by means of the innumerable cilia which cover them.

FIG. 18. — Bilharzia Hæmatobia Ovum.

Importance.—The parasite is acquired in some way from water, chiefly in Egypt and South Africa. Many cases occurred during the late war. The diagnosis of the cause of the hæmaturia depends on finding the ova.

10. **Prostatic Threads.**—If the urine be poured into a glass cylinder, these may be seen by the naked eye floating about as fine threads; being composed of mucus, they appear structureless under the low power.

Importance.—They point to previous gonorrhœa.

11. **Mucus.**—This is a normal constituent of many urines, particularly in women. Under the microscope it appears as structureless wisps and bands, in which

may be embedded crystals, leucocytes, and so forth. The wisps stain deeply with the methylene blue.

Importance.—In most cases mucus has no pathological significance. If present in great abundance it suggests irritation of the urinary passages, the nature of the irritant being indicated by the calcium oxalate crystals, pus cells, or other feature of the urine that may be noted at the same time. It is easy, before experience is acquired, to mistake mucus for renal tube-casts, and this in two ways: First, parts of the mucus often have a cylindrical form—'mucous cylindroids'; secondly, if in pressing down the cover-slip on to the slide the former slips slightly along the latter, the mucus becomes pressed out lengthways in straight cast-like streaks; the latter will at once be detected for what they are, because there will be quantities of them, and they will be all lying with their long axes parallel to one another.

III. TESTS FOR SUBSTANCES IN SOLUTION.

1. **Albumin.**—Filter the urine if it be turbid; it is essential for the following tests that it should be clear.

Heller's Nitric Acid Test.—Pour 1 inch of non-fuming nitric acid into a test-tube; hold the latter slanting, and allow urine to flow gently from a pipette on to the surface of the acid. Watch the line of junction; an opaque white ring will appear at once if much albumin be present, more slowly with less. A brownish-red ring which often appears also is quite

independent of the presence of albumin, and results from the oxidation of indican by the nitric acid.

Fallacies.—(1) If fuming nitric acid be used, the urea will be decomposed, giving off bubbles of CO_2 and N_2. This will cause the urine to mix with the acid, so that the line of junction is destroyed, and even though albumin be present, it may not be possible to distinguish the white ring.

(2) Albumose will give a similar white ring; but it disappears on warming, to reappear on cooling, whereas that due to albumin remains on warming.

(3) If the urine be concentrated, urea nitrate may form a white ring. Dilute the urine with an equal bulk of water, and repeat the test. Urea nitrate will now remain in solution.

(4) After the administration of drugs containing resins—*e.g.*, copaiba—a white ring appears with nitric acid; it is more diffuse than that of albumin. It is due to precipitation of the resin, which will redissolve on adding a little alcohol.

Test by Heating.—Fill a test-tube three parts full of urine; note the reaction to litmus paper. If alkaline, just acidify with acetic acid; the object of this is to prevent the formation of uncoagulable alkali-albumin on warming. Acetic acid is used because a stronger, such as nitric or hydrochloric, would form acid-albumin, which is likewise uncoagulable by heat. Boil the upper 1 inch thoroughly, and look through the test-tube at a dark background. If the boiled inch is as clear as the unboiled urine, no albumin is present; if it has become cloudy, the pre-

cipitate may be due to one or more of three things—namely:

(i.) Albumin.
(ii.) Phosphates.
(iii.) Nucleo-proteid.

To decide between these, first add 2 or 3 drops more acetic acid; if the cloud clears up entirely, it was due to phosphates; if it remains, albumin or nucleo-proteid, or both, are present. Now add 1 drop of strong nitric acid; this will clear up the cloud if it is due to nucleo-proteid, and if the cloud persists, albumin is present.

Fallacies.—(1) Phosphates (see above).

(2) Nucleo-proteid (see above).

(3) If the urine be not acid, alkali-albumin may be formed, and no cloud appear, though albumin be present.

(4) When the amount of albumin present is small, it may be missed if the boiling be not continued for half a minute or more.

The heat test is the best for albumin, and has the fewest fallacies.

The Salicyl-Sulphonic Acid Test.—It is essential to have a particularly small test-tube. Into this put 30 drops of the urine, and add a few drops of a saturated aqueous solution of salicyl-sulphonic acid. If no precipitate occurs, no proteid is present. If there is a precipitate, boil the tube; if the liquid in it becomes clear again, the precipitate was due to proteoses and not albumin; if the precipitate persists, it is due to albumin; indeed, with albumin it increases by coagulation on heating. The main advantage of the test is that it saves time when many examinations

have to be made, as in hospital work; for if no precipitation occurs with the reagent in the cold, no heating is required; the reagent is very stable, and no particular care is required as to the exact amount of it used for each test.

There are many other tests for albumin, such as those with potassium ferrocyanide, picric acid, and so forth, but the above are far more reliable and delicate than any of the others.

Importance of Albumin.—The presence of albumin in the urine is seldom without significance, though far from always meaning Bright's disease. In women it may be an accidental accompaniment at the menstrual period, or arise from vaginitis or some uterine discharge; in men it may be temporary after seminal emission; and in boys a so-called 'functional' albuminuria is almost common about the time of puberty. With blood or pus albumin is always present, but pus accounts for far less albumin than might at first be expected. It is frequent with heart failure, and may result from any cause of low arterial blood-pressure. Abdominal tumours, ascites, and inferior vena cava thrombosis may cause albuminuria by obstructing the outflow from the renal veins. It is not uncommon in the severe anæmias, and accompanies lardaceous disease of the kidneys. Only when casts are present as well as albumin can Bright's disease be diagnosed with certainty. Microscopical examination of the urinary deposit should never be omitted in a case of albuminuria.

2. **Blood.**

The Guaiacum Test.—To 1 inch of urine in a

test-tube add 1 drop of tincture of guaiacum; the resin forms a white precipitate. Pour on to the surface 1 inch of ozonic ether. If blood be present, it and the ozonic ether together oxidize the guaiacum, and a blue colour appears at the junction of the fluids.

Fallacies.—(1) Pus gives a similar colour, but it is more green than blue, and appears more slowly. Pus will give the colour with the guaiacum alone.

(2) Iodides in the urine give a similar blue colour, but it appears more slowly than with blood.

(3) Adventitious organic matter due to vomit or sputum may also give the blue colour. The fallacy may be avoided by testing a catheter specimen.

(4) It is always well to be sure that the tap-water does not itself give the blue colour. In places where the tap-water is not to be used for drinking purposes, it is common for enough organic matter to be present in it to give a very distinct blue ring with the ozonic ether and tincture of guaiacum test.

Heller's Test.—Phosphates have an affinity for blood pigment, and carry it down with them. Render some urine in a test-tube strongly alkaline with caustic soda, and boil it; if blood be present, the deposit of earthy phosphates is brownish-red.

Fallacies.—(1) Earthy phosphates may be deficient in the urine, and no deposit result. To obviate this, add 2 drops of calcium chloride solution.

(2) Certain drugs—rhubarb, senna, santonin—give a similar reaction.

(3) The test is seldom used clinically. Its main interest lies in the fact that when phosphates are spon-

taneously deposited in a urine containing blood, they carry the blood pigment with them, and appear dingy brown instead of white (p. 4).

The Spectroscope Test.—Even a small pocket spectroscope is expensive, so that the test is little employed. When the microscope reveals red corpuscles, it is not needed; but in cases of hæmoglobinuria it is valuable in confirming the presence of blood pigment. Filter the urine if it be not clear; dilute it with water if it absorb too much light. With the spectroscope look at a bright light through a test-tube filled with the urine. If recent blood be present, the two absorption bands of oxyhæmoglobin will be noted (Fig. 19), one at the junction of the yellow with the

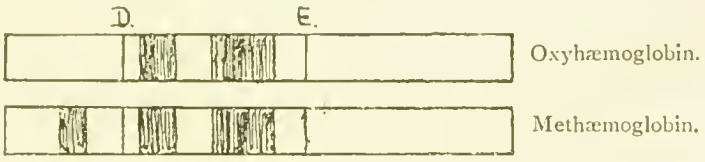

FIG. 19.—Spectra of Blood in Urine.

green, the other in the middle of the green rays of the spectrum. Blood that has been longer mixed with the urine gives the three bands of methæmoglobin (Fig. 19), one in the red in addition to the two of oxyhæmoglobin.

Fallacies.—None, except that the test requires care and skill in carrying it out. Other substances have spectroscopic bands, but none exactly like the above.

The Microscope Test.—Examine the centrifugalized deposit for red corpuscles; their presence is the best proof of hæmaturia.

Fallacies.—(1) In alkaline urine or in urine of very low specific gravity the red corpuscles may swell up and disappear.

(2) In paroxysmal hæmoglobinuria and in black-water fever blood pigment is present, but no red corpuscles.

Importance of Blood in the Urine.—Except in women at the menstrual period, the presence of blood is always pathological. Laboratory tests cannot by themselves decide what part of the genito-urinary tract the blood comes from; but when the blood is intimately mixed with the urine, the source is usually the kidney, whilst when it comes chiefly at the beginning or the end of micturition, the source is elsewhere. Methæmoglobin in fresh urine indicates renal hæmorrhage rather than vesical. Hæmaturia does not always indicate a lesion in the urinary tract, for it may occur in the blood diseases, such as leuchæmia or purpura.

3. **Pus.**—The deposit is opaque and white; in small quantities it may be mistaken for mucus; in larger for phosphates or for colourless urates. Urates disappear on warming, pus remains; phosphates increase with heat, but clear up with acetic acid. Mucus and pus can be distinguished by the following tests:

The Liquor Potassæ Test.—To 1 inch of the suspected deposit in a test-tube add 1 inch of liquor potassæ; pour the mixture from one test-tube into another. Pus will have partially dissolved and become 'ropy' and gelatinous.

Fallacy.—The test will not detect small quantities of pus.

The Ozonic Ether Test.—To 1 inch of the deposit in a test-tube add 1 inch of ozonic ether. On gently shaking, numbers of small bubbles of liberated oxygen will be seen rising through the fluid.

Fallacies.—(1) Blood also causes bubbling with ozonic ether.

(2) Normal urine causes a few bubbles to come off, so that the test will not detect small quantities of pus.

The Guaiacum Test.—This is performed as for blood. Even without adding ozonic ether, a green-blue colour appears after a few minutes.

Fallacies.—Blood, iodides, saliva (p. 28).

The Microscope Test.—Under $\frac{1}{6}$-inch objective pus corpuscles appear as leucocytes which have undergone granular or fatty degeneration. Their nuclei can be brought out by adding a drop of acetic acid (Fig. 15, p. 16).

Fallacy.—If it be remembered that a few leucocytes may be found in normal urines, there is no fallacy. The presence of numerous leucocytes in the urine indicates pus. The microscope is the only satisfactory test for it.

Importance of Pus.—It is always pathological. Small quantities of pus derived from the urethra or vagina may occur in acid urine. Much pus in an acid urine indicates renal suppuration, in which case the acidity is maintained by healthy urine from the sound kidney. If both kidneys be affected, or if there be cystitis, the purulent urine will be alkaline and probably ammoniacal.

4 **Sugar.**—Two sugars may occur in urine,

glucose and lactose.* The following tests are for glucose:

Fehling's Test.—Fehling's solution is made by mixing equal parts of the following fluids:

Copper Sulphate Solution.

Crystallized copper sulphate ..	34·64	grammes
Distilled water up to	500	c.c.

Rochelle-Salt Solution.

Potassium hydrate	125	grammes
Sodium potassium tartrate (Rochelle salt)	173	,,
Distilled water up to	500	c.c.

The copper sulphate solution is pale green, that of Rochelle salt colourless. On mixing the two the potassium hydrate converts the copper sulphate into cupric hydrate, and the latter is kept in solution by the Rochelle salt, giving a deep blue transparent fluid. The separate fluids keep good any length of time; mixed, they slowly decompose. They should therefore be kept in separate bottles and mixed as required. To perform the test, boil 1 inch of Fehling's solution in a test-tube; if good, it remains clear. In another test-tube boil 1 inch of urine. Pour the hot urine into the hot Fehling's solution. If glucose be present it will reduce the cupric hydrate to cuprous oxide, which comes down as an orange precipitate.

Fallacies.—(1) Lactose reduces Fehling's solution, but may be distinguished by the fermentation and the phenyl-hydrazine tests (p. 35).

(2) Slight reduction of Fehling's solution, causing

* It is for larger books than this to discuss pentoses and other rarities.

first a greenish colour of the solution, and then a dark greenish-yellow precipitate, may be caused by other reducing substances in the urine, namely :

Uric acid.	Creatinin.
Hippuric acid.	Glycuronic acid.
Xanthin.	Glycosuric acid.
Hypoxanthin.	Alkapton.

And the products of certain drugs :

Chloral.	Carbolic acid.
Chloroform.	Salicylates.
Glycerine.	Urotropine.

None of these ferment. Error from these sources is not likely to occur if the two fluids be boiled separately and then mixed. Glucose and lactose alone will give the reduction without further boiling.

(3) Albumin interferes with Fehling's test. If it be present, add acetic acid, boil, filter off the coagulated albumin, and test the filtrate.

(4) In ammoniacal urines the free ammonia may prevent precipitation of cuprous oxide. Before testing with Fehling's solution, boil well to expel the ammonia.

Trommer's Test. — Trommer's solution is a mixture of copper sulphate and potassium hydrate. It differs from Fehling's in not containing Rochelle salt. The test is performed in precisely the same way and has the same fallacies.

Böttger's Test.—The reagent used is the following :

Caustic soda	10 grammes
Sodium potassium tartrate (Rochelle salt)	4 ,,
Bismuth subnitrate	2 ,,
Distilled water	100 c.c.

To 2 inches of urine in a test-tube add 5 drops of the reagent. Boil for five minutes. If glucose be present the bismuth is reduced, and a fine black precipitate of bismuth suboxide appears.

Fallacies.—Lactose and glycuronic acid also give the test; but uric acid, creatinin, and xanthin bases, which give some reduction with Fehling's test, give none with Böttger's.

The Picric Acid Test.—To 1 inch of urine in a test-tube add $\frac{1}{4}$ inch of saturated solution of picric acid and 5 drops of caustic potash. Boil. If glucose be present, the solution darkens in colour, becoming first dark brown and then almost black from the reduction of picric to picramic acid.

Fallacies.—(1) Lactose gives the test also.

(2) In normal urine creatinin causes some darkening, so that as a sugar test picric acid is not very delicate.

(3) Impure picric acid darkens when boiled with caustic potash. The solution should therefore be tested before it is added to the urine.

Moore's Test.—Mix 1 inch of urine with 1 inch of liquor potassæ in a test-tube and boil. If sugar be present a ruddy brown colour develops, going on to dark brown and then almost black.

Fallacies.—(1) The test will not detect small quantities of sugar.

(2) Carboluria, alkapton, and indican also cause darkening with potash.

The Phenyl-hydrazine Test.—To 2 inches of urine in a test-tube add as much phenyl-hydrazine chloride as will lie easily on a threepenny-piece, and

a rather larger quantity of sodium acetate. Stand the test-tube in boiling water for twenty minutes, then allow it to cool. If glucose be present, phenyl-glucosazone crystals are formed; these may be pipetted off and examined under the low power; they are very characteristic fans and sheaves of long bright yellow needles (Fig. 20).

FIG. 20.—Phenyl-glucosazone Crystals.

Fallacies—(1) Lactose gives yellow needles of phenyl-lactosazone, but they are smaller, and arranged less as fans and sheaves than as spheres with radiating spines (Fig. 21).

(2) If the crystals are abundant, there is not the least doubt about the presence of glucose; if they are present in small numbers only, however, and perhaps not quite typically formed, there may be a doubt as to whether the atypical crystals may not be due to glycuronic acid.

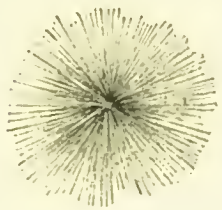

FIG. 21.—Phenyl-lactosazone Crystals.

The Fermentation Test.—Triturate a piece of good yeast, the size of a small marble, with 250 c.c. of urine. Fill a Southall's ureometer (Fig. 27, p. 53) with the mixture, and allow it to stand in a warm place for twenty-four hours. Glucose, if present, will ferment, and the CO_2 given off will collect as a bubble at the top of the closed limb of the ureometer.

Fallacies.—None. No other reducing body in the urine will ferment with yeast.

Summary of Sugar Tests.

For Glucose.	*For Lactose.*
Fehling's test serves all ordinary purposes.	Gives Fehling's test.
Phenyl-hydrazine test is a good confirmation.	Gives crystals with the phenyl-hydrazine test different to those of glucose.
Fermentation test absolute proof of the presence of glucose.	Will not ferment with yeast.

Moore's, Trommer's, Böttger's, and the picric acid tests are confirmatory.

Importance of Sugar in the Urine.—It is important to know if the sugar be glucose. Lactose is not pathological, but is closely related to pregnancy in women, occurring both before and after parturition. Glucose in appreciable quantities is pathological. Its presence may be temporary, as after alcoholic excess, an injury to the head, or a vascular lesion of the brain. It may be intermittent in the gouty or alimentary glycosuria of elderly people, or it may indicate a more advanced stage of diabetes mellitus, in which case it is often associated with acetone and diacetic acid.

5. Acetone.

The Sodium Nitroprusside Test.—To 1 inch of urine in a test-tube add 1 inch of caustic soda and $\frac{1}{2}$ inch of 1 per cent. solution of sodium nitroprusside. A ruby red colour appears. Add acetic acid. If acetone be present the colour deepens to a rich port-wine colour.

Fallacies.—(1) If potash or ammonia be used instead of soda, the test may fail.

(2) Creatinin gives a similar ruby red with the nitroprusside and caustic soda, but on adding acetic acid the colour, instead of deepening, disappears.

The Iodoform Test.—To 1 inch of urine in a test-tube add 5 drops of caustic potash; warm to body heat; add iodine solution until the liquid is yellowish brown; warm gently. If acetone be present iodoform will form, and it may be recognized by its odour or by its yellow crystals.

Fallacy.—It is seldom possible to be sure that iodoform has been formed unless the acetone has been obtained in concentrated form by distilling the urine, collecting the first few c.c. that come over, and then applying the test to the distillate. This is scarcely possible in ordinary practice, so that although with distillation the iodoform test is the best, for ordinary clinical work the sodium nitroprusside test is most used.

Importance of Acetone in the Urine.—Apart from sugar, acetone has been found in lobar pneumonia, tetany, gastric ulcer, starvation, and other conditions; associated with glycosuria, acetone indicates diabetes mellitus, with acidosis and consequent liability to coma. Diabetes without acetonuria is a much more favourable stage than diabetes with acetonuria.

6. **Diacetic or Aceto-acetic Acid.**—To 1 inch of urine in a test-tube add liquor ferri perchlor. (B.P.) drop by drop. A white precipitate of iron phosphate forms. Later, if aceto-acetic acid be present, the liquid becomes deep purple-red, but is decolorized by warming.

Fallacies.—If carbolic acid, salol, salicin, salicylates,

are being taken, the urine contains phenyl compounds which give a similar reaction with ferric chloride. The colour due to these causes does not disappear on warming.

Importance.—Similar to that of acetone. β-oxybutyric acid is a precursor of acetone and diacetic acid, all three being progressive stages in the end-products of a pathological metabolism of proteid. They are all factors in the dangerous acidosis of diabetes. β-oxybutyric acid is nearly always present along with acetone, but there is no easy test for it. For clinical purposes, therefore, it is sufficient to test for the latter.

7. **Glycuronic Acid.**—There is no good clinical test for this acid; its main importance is that it may be mistaken for sugar because it reduces Fehling's and Böttger's solutions. But *it will not ferment.* It occurs in normal urine to a small extent, and is increased after giving chloral, butyl chloral, nitrobenzol, camphor, and after chloroform narcosis.

8. **Bile Pigments.**—The colour varies from brownish yellow to dark green, and may be simulated by carboluria, alkaptonuria, hæmaturia, indicanuria, melanuria; but if the urine be shaken up the colour of the froth also is greenish when bile pigments are present—not so in the other cases.

Gmelin's Test.—Upon a white porcelain slab put 1 drop of the urine, and close to it a drop of fuming nitric acid. At their point of coalescence a play of colours—yellow, red, green, blue, yellow—will occur if bile pigments be present. The test may also be performed in a similar way on filter-paper; or, if 1 inch of nitric acid be put into a test-tube, and the urine

carefully poured on to its surface, the play of colours is readily seen at the junction of the two fluids.

Fallacy.—None.

The Iodine Test.—Take 1 inch of urine in a test-tube; gently pour 10 per cent. solution of iodine in alcohol on to its surface. In the presence of bile pigments a bright green ring appears between the two liquids.

Fallacy.—The test is not so delicate as Gmelin's.

Importance of Bile Pigments in Urine.—They indicate jaundice, but not its cause.

9. **Bile Salts.**

Pettenkofer's Test.—When to a solution of bile salts in a test-tube 1 c.c. of strong solution of cane-sugar is added, and strong sulphuric acid drop by drop, holding the end of the test-tube in running water to keep it cool, a rich crimson colour appears.

This test, however, is not applicable to fresh urine. Bile pigments may be abundant, and yet Pettenkofer's test may fail to show bile salts. It is seldom of any clinical importance to know whether bile salts as well as bile pigments are present; but if they are to be tested for, it is essential to extract them from the urine with chloroform, and then apply Pettenkofer's test to the chloroform extract.

The Sulphur Test.—Fill a test-tube to the top with urine; sprinkle finely powdered commercial sulphur on to its surface. Whereas in healthy cold urine the sulphur floats, even minute traces of bile salts so alter the surface tension that the sulphur sinks.

Fallacies.—(1) If the urine be warm, the sulphur sinks even in the absence of bile salts.

(2) The test is almost too delicate. By it all sorts of urines are shown to contain traces of bile salts, so that the very delicacy of the test detracts from its clinical value.

Importance of Bile Salts.—Bile salts are not known to give evidence which is not equally afforded by the bile pigments.

10. **Chlorides.**—To 1 inch of urine in a test-tube add 5 drops of nitric acid and 2 drops of silver nitrate solution. Chlorides give a dense white precipitate. The nitric acid is added first to keep the phosphates and sulphates in solution.

Fallacy.—Albumin and albumose, if present, are precipitated by the nitric acid.

Importance.—Some deficiency of chlorides occurs in many fevers, but in lobar pneumonia they may be almost or quite absent, assisting in diagnosis from empyema or simple pleurisy. Presence of chlorides does not exclude lobar pneumonia, and they may be absent in other conditions; for example, suppurative peritonitis, or in absolute starvation. In chronic nephritis there is a great delay in the elimination of chlorides, so that examination for these may afford evidence as to the degree of renal insufficiency.

11. **Indican.**—To 2 inches of urine in a test-tube add 1 inch of strong hydrochloric acid and 5 drops of fuming nitric acid; allow to cool; add ½ inch of chloroform, and shake up thoroughly. If indican be present, the chloroform, when it has again sunk to the bottom of the test-tube, will be tinged either blue or red. The principle of this test is to oxidize the indican. Nitrohydrochloric acid is a convenient agent for this pur-

pose; freshly prepared bleaching powder is sometimes used instead. The chloroform is used merely to dissolve out the oxidized indican, in order that the colour may be more easily seen than it can be in the urine. Whether the result is blue or red seems to depend upon the temperature the solution reaches; the less it is heated the more likely is the colour to be blue.

Fallacies.—(1) Albumin interferes with the test. It should, if present, be first removed by acidifying the urine with acetic acid, boiling, and filtering off the coagulated proteid.

(2) Iodides, if present, will colour the chloroform blue.

Importance.—'Indican' is a mixture of potassium indoxyl and skatoxyl sulphates, derived from the indol and skatol which arise from intestinal decomposition. Indicanuria thus points to putrefaction in the intestine from chronic constipation, from acute or ulcerative colitis or enteritis, and other similar conditions.

12. **Urobilin.**—Examined with the spectroscope, an absorption band between the green and blue is typical of urobilin (Fig. 22). To 1 inch of urine in a

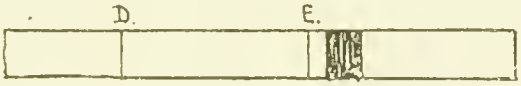

FIG. 22.—Urobilin Spectrum.

test-tube add ½ inch of strong ammonia, and then zinc chloride solution drop by drop; filter. If urobilin be present the filtrate will be fluorescent, and will show the spectroscopic band more markedly than before.

Importance.—In healthy urine the chief pigment is urochrome, not urobilin. The latter is a pathological

pigment. It may occur in small amounts in many febrile diseases, but it is found most particularly when the liver is diseased, especially in cases of hepatic cirrhosis and in pernicious anæmia.

13. **The Diazo-Reaction.** — Ammonia and the following two solutions are needed; each should be fresh:

Sulphanilic Acid Solution.

Hydrochloric acid	5 c.c.
Distilled water	100 ,,
Sulphanilic acid to saturation.	

Sodium Nitrite Solution.

Sodium nitrite	0·5 gramme
Distilled water	100 c.c.

To 1 inch of urine in a test-tube add 1 inch of sulphanilic acid solution and 5 drops of the sodium nitrite. Add ammonia till alkaline, and shake thoroughly. The test is positive if the liquid becomes port-wine colour *and the froth rose red.* The rose colour of the froth is essential.

Importance.—The reaction used to be used in the diagnosis of typhoid fever, but it has gone out of fashion as being much less valuable than Widal's test (p. 92). A positive diazo-reaction in phthisis is a bad sign; but if the reaction has at one time been found, and later has disappeared, it indicates improvement in the patient.

14.—**Albumose.**—First remove albumin by acidifying the urine with acetic acid, boiling and filtering. On to the surface of 1 inch of nitric acid in a test-tube gently pour some of the cooled filtrate. If albumose be present, a white ring will appear at the junction of

the fluids. The ring will disappear on warming, to reappear again on cooling.

Importance.—Albumosuria occurs to a slight degree in many fevers, notably pneumonia, but it has no known significance. It also occurs when there are collections of pus under pressure, as in pelvic or appendicular abscess or empyema. In large quantities it appears to be pathognomonic of endosteal sarcoma of bone.

15. **Nucleo-proteid.**—The chief interest that nucleo-proteid has for the clinician is that it may, if not recognized, lead to an erroneous diagnosis of albuminuria when the heat test alone is used (p. 26). It is therefore of great importance in examinations for life insurance. Almost all urines contain a trace of nucleo-proteid. Some contain much more than a trace, in which case further examination will show that there is something in the urine which irritates the urinary passages—calcium oxalate, for example (p. 7).

Nucleo-proteid is precipitated by acetic acid. If a trace of albumin is present, together with excess of nucleo-proteid, and it is desired to find out the relative proportions of each, a good plan is as follows: Into each of two test-tubes, A and B, pour an equal quantity of the urine, and strongly acidify each with the same number of drops of acetic acid. Leave A unboiled, but boil B. When B has cooled, compare the degree of opacity in the two. In A the precipitate is due to nucleo-proteid only, in B to nucleo-proteid together with the albumin coagulated by heat. If any albumin is present, therefore, the cloud in B must be more than that in A.

16. **Carboluria, Alkaptonuria, and Melanuria.**—These three conditions account for various greenish-brown, greenish-black, brownish-black, and blackish tints that are sometimes observed in urines. They may be mistaken for bile or blood pigments. The urine is frequently normal in colour when passed, but darkens when exposed to the air. In carboluria this is due to oxidation of hydrochinone and pyrocatechin; in alkaptonuria to homogentisic acid; in melanuria to melanogen, which becomes melanin on standing. The darkening is in each case accelerated by adding caustic potash. The following reactions will indicate the probable nature of a given case:

In Carboluria.—Ferric chloride gives a white precipitate of phosphates and a *purple* solution. Bromine water gives a dense pale yellow precipitate of tribromophenol.

In Alkaptonuria.—Fehling's solution gives a reduction, but the urine does not ferment with yeast. Dilute solution of ferric chloride gives a *green* colour, which rapidly disappears, returning with the addition of more of the reagent.

In Melanuria.—Ferric chloride: the precipitate of phosphates carries down the melanin as a dark grey deposit, soluble in excess of ferric chloride. Bromine water gives a dense yellow precipitate, which slowly blackens. There is no reduction with Fehling's solution. Caustic potash, sodium nitroprusside, and acetic acid give a deep blue colour.

Importance.—Carboluria is by no means synonymous with carbolic acid poisoning. Some patients are so susceptible that carbolic acid lotion or dressings,

carbolic acid or salicylates by the mouth, may cause the condition without there being any other symptoms. Nevertheless, it is wise to desist from the use of the particular drug or application which causes carboluria in any given case.

Alkaptonuria is the result of certain peculiarities in proteid metabolism quite compatible with good health. Few individuals present the abnormality.

Melanuria may occur in patients suffering from melanotic sarcoma, but it is not constant in such cases, and it is sometimes marked in other wasting diseases.

17. **Lead.**—Add 1 gramme of ammonium oxalate to 150 c.c. of urine; immerse a strip of bright magnesium wire in it for twenty-four hours. Lead, if present, will form a deposit on the magnesium. Dissolve this in dilute nitric acid. Test the solution for lead as follows:

(1) A drop of HCl added gives a dense white precipitate of $PbCl_2$.

(2) Chromate of potash gives a bright yellow precipitate of lead chromate.

(3) Ammonium sulphide in excess gives a black precipitate of lead sulphide.

(4) Potassium iodide gives a bright yellow precipitate of lead iodide.

Importance.—Lead is excreted in the urine after the administration of potassium iodide in cases of plumbism, and sometimes an otherwise doubtful diagnosis may be cleared up by discovering lead in the urine.

18. **Some Drug Reactions in the Urine:**

Antifebrin.—Extract the urine with chloroform.

To the extract add nitrate of mercury solution and warm. A green colour indicates that antifebrin is being taken.

Antipyrine.—The urine may be red, suggesting blood, but the guaiacum reaction is negative. With ferric chloride, a brownish-red colour forms.

Chloral and Chloroform cause increase of glycuronic acid. With Fehling's solution there will be some reduction, but there will be no fermentation with yeast.

Copaiba.—With nitric acid a white ring, which might be mistaken for albumin. Ring redissolved by alcohol. Add 2 drops of HCl to urine in a test-tube. Copaiba gives a pink colour.

Iodides.—With guaiacum, blue colour; may suggest blood. Add HCl and shake up with chloroform; the latter takes up the liberated iodine, becoming violet; indican gives a similar reaction, but requires HNO_3 as well as HCl.

Rhubarb. — Reddish-brown urine may suggest blood. No guaiacum reaction. With caustic potash red colour deepens; on now adding HCl it becomes yellow.

Salicylates, Salol, and Carbolic Acid.—With Fehling's solution partial reduction on continued boiling; with ferric chloride a purple colour, not disappearing on warming, and thus unlike diacetic acid.

Santonin.—Bright yellow urine. Caustic potash turns it bright pink.

Sulphonal.—Sometimes causes hæmatoporphyrinuria, the urine being port-wine coloured, suggesting

blood, and yet not giving the guaiacum test. Add 2 drops of acetic acid to some urine, and shake it up with an equal volume of ether. This extracts the pigment, which, examined spectroscopically, gives the four-banded spectrum of alkaline hæmatoporphyrin (Fig. 23). When HCl is added it changes to that of

FIG. 23.—Hæmatoporphyrin Spectra.

acid hæmatoporphyrin—namely, a band in the orange and another between the yellow and green (Fig. 23).

Urotropine.—The addition of caustic soda and then of solution of phloroglucin gives a deep red colour. The urine also gives some reduction with Fehling's solution.

IV. METHODS OF ESTIMATING VARIOUS CONSTITUENTS OF URINE.

1. Estimation of Albumin.

Esbach's Method.—An Esbach's albuminometer (Fig. 24) and Esbach's fluid are needed. The former is a special test-tube, graduated in its lower part by marks which correspond to parts per thousand, and with two other marks, U and R respectively.

Esbach's fluid is as follows:

Picric acid	10 grammes
Citric acid	20 ,,
Distilled water to	1,000 c.c.

Fill the tube with urine to the mark U, and add reagent to the mark R; cork, and gently invert several times. Stand vertically for twenty-four hours. The mark then corresponding to the upper level of the precipitate gives the parts per thousand of albumin.

Fig. 24. — Esbach's Albuminometer.

Fallacies.—Occasionally the albumin floats instead of sinking. In that case shake the tube up again and leave for another twenty-four hours.

The test is one of sedimentation, so that if left for more or less than twenty-four hours the reading will be proportionately less or more than it should be.

The higher graduations become closer and closer together, so that the difference for each part per 1,000 over 5 is difficult to measure accurately. If more than 5 parts per 1,000 are present, dilute the urine once, twice, thrice, as may be necessary, and multiply the amount then found by 1, 2, or 3 respectively.

The picric acid also precipitates pigments and aromatic compounds. The amount of these is negligible in proportion to large amounts of albumin, but with a reading less than 1 part per 1,000 the error due to pigments is great. The readings are therefore accurate only between 1 per 1,000 and 5 per 1,000, and only after standing exactly twenty-four hours.

Roberts's Method.—This depends on the fact that it requires three minutes for the white ring with nitric acid to appear when there is 1 part of albumin in 30,000; no special apparatus is required. Dilute the

urine ten, twenty, thirty times, and so on, and after each dilution test with nitric acid. Suppose the white ring required three minutes to appear after thirty times dilution, there would be 30 parts of albumin per 30,000, or 1 part per 1,000.

2. Estimation of Sugar.

Pavy's Method.

Pavy's Solution.

Copper sulphate	4·16 grammes
Rochelle salt	20·4 ,,
Caustic potash	20·4 ,,
Strong ammonia	300 c.c.
Water to	1,000 ,,

10 c.c. are reduced by 0·005 gramme of glucose.

Pavy's solution differs from Fehling's in two respects—viz., it is ten times as dilute, and it contains ammonia; the latter is to prevent the precipitation of cuprous oxide. The fresh solution is blue; when reduced, colourless. Fill a burette with the urine diluted 1 in 20. Put 10 c.c. Pavy's solution and 20 c.c. water into a 200 c.c. flask fitted with a cork bored with two holes. Through one hole pass the nozzle of the burette, through the other a glass tube 4 inches long, which reaches just into the flask at one end, and is open to the air at the other (Fig. 25). Heat the flask until the Pavy's solution just boils; keep it simmering; turn the stop-cock of the

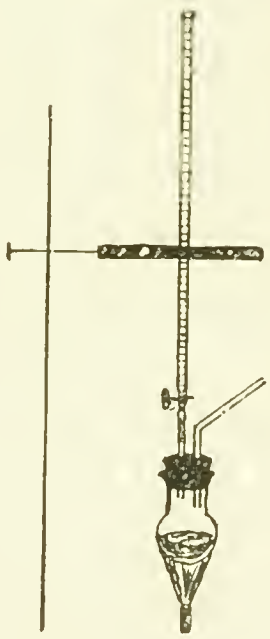

FIG. 25.—Pavy's Estimation of Sugar.

burette so that the diluted urine drops in steadily at the rate of 3 drops per second. Do not alter this rate of dropping, but watch the colour of the fluid in the flask; it will presently fade. When the Pavy's solution is just colourless, turn the stop-cock off, and read off the amount of urine added. The calculation is as follows: The 10 c.c. of Pavy's solution require 0·005 gramme of glucose for complete reduction. Suppose 3 c.c. of the twenty times diluted urine were added;

$\therefore$ in 3 c.c. of twenty times diluted urine there are 0·005 gramme of glucose.

$\therefore$ in 1,000 c.c. of twenty times diluted urine the reare $\frac{0{\cdot}005 \times 1{,}000}{3}$ grammes of glucose.

$\therefore$ in 1,000 c.c. of original urine there are $\frac{0{\cdot}005 \times 1{,}000 \times 20}{3}$ grammes of glucose.

$= 33{\cdot}3$ per 1,000.

The method is liable to considerable personal error; it gives strictly comparable results, however, if always performed by the same person and in the same way.

If the flask be not corked as described, the ammonia rapidly boils off, and a thick yellow deposit of cuprous oxide comes down.

The urine should always be diluted to such an extent that not less than 1 c.c. and not more than 3 c.c. are required to decolourize the 10 c.c. of Pavy's solution. (N.B.—After the estimation, the blue colour will return to the reduced Pavy's solution, owing to reoxidation of the cuprous oxide in the flask.)

Gans' Method.—This is less accurate than Pavy's, but may be employed in general practice, as it is less complicated. It depends on the volume of CO_2 evolved on fermentation with yeast. To 10 c.c. of urine add a small quantity of finely triturated yeast; dilute to 100 c.c. with water; mix thoroughly. Transfer 10 c.c. to the bulb of a Gans' apparatus (Fig. 26), and insert the stopper. The graduated vertical limb is open at the top, and there is an arrangement, easily understood when the apparatus is seen, for adjusting the level of the fluid to the zero mark. Stand in a warm place till next day. The CO_2 evolved in the bulb will have forced the fluid up in the vertical limb, and the graduation corresponding to its upper level gives the parts per cent. of sugar in the original urine. The graduations on the tube having been made experimentally with known percentages of glucose, it does not matter that a certain proportion of the CO_2 escapes from the open end.

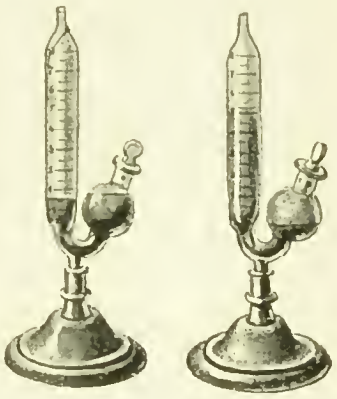

FIG. 26.—Gans' Apparatus.
A, before fermentation; B, after.

The method avoids error from presence of other reducing bodies than sugar in the urine.

The chief inaccuracy is the variation in volume of gas in the bulb with changes in temperature and barometric pressure.

Specific Gravity Method.—This is easy, and affords approximate results. It is very useful in

private practice. Mix some finely triturated yeast with a quantity of urine. Take the specific gravity of the mixture. Stand in a warm place for twenty-four hours, and take the specific gravity again. Glucose will have fermented, and the specific gravity will have fallen. Every degree of specific gravity lost corresponds to 1 grain per ounce of glucose. For example:

Specific gravity—before fermentation	1040
,, ,, after ,, ...	1022
Grains per ounce of glucose ..	18

3. Estimation of Urea.

Sodium Hypobromite Method with Southall's Ureometer.

Hypobromite Solution.

Caustic soda	100 grammes
Water	250 c.c.
When cool add bromine	25 ,,

Sodium hypobromite (NaBrO) is formed, and caustic soda is in excess.

Fill the bulb of the ureometer (Fig. 27, A) with this solution, and tilt the apparatus so as to displace all the air in the closed vertical limb. Measure off 1 c.c. of urine with the pipette (Fig. 27, B). A good way of doing this is to expel *all* the air from the elastic nipple, plunge the end of the glass pipette into the urine, and release the nipple so that urine is drawn up beyond the mark. Raise the pipette out of the urine, and by gentle pressure on the nipple expel the excess down to the mark 1; then plunge the tip into clean water and release the nipple again. It does not matter that the measured 1 c.c. of urine will thus be slightly diluted.

Now insert the end of the pipette through the bulb of the ureometer till it reaches the bottom of the vertical limb; slowly compress the nipple, expelling first the water and then the urine into the hypobromite solution. As soon as the urine mixes with the hypobromite, bubbles of gas come off, the urea being decomposed into CO_2, H_2O, and N_2. The CO_2 is dissolved by the excess of soda; the H_2O condenses;

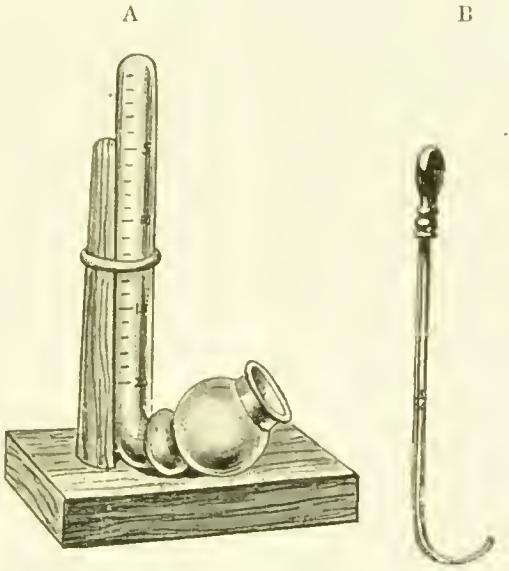

FIG. 27.—Southall's Ureometer (A), and Pipette (B).

only the N_2 rises to the top of the vertical limb. Allow the froth to settle down; read off the graduation corresponding to the lower level of the gas. There are two sets of graduations, one showing parts per cent., the other grains per ounce.

Importance.—A healthy man excretes about 400 grains, or 30 grammes, of urea in twenty-four hours; but there are wide limits, according to the food taken.

In the majority of cases a small percentage of urea means one of two things: either little nitrogenous food is being taken or the urine is very dilute. This is apparently as true in Bright's disease as in other illnesses. In certain fevers more urea is excreted than corresponds to the nitrogen in the food, owing to breaking-down of the patient's own tissues. This may also be the case in diabetes, though no deductions can be drawn unless the nitrogen in the food is also known. In surgery much stress is laid upon the amount of urea as an indication for or against operating in cases of tuberculous kidney, stone in the kidney, and so forth. When the total urea in twenty-four hours' urine is less than 100 grains, it is held to be inadvisable to operate. This is probably the case, but it must not be thought that it is an indication that the kidneys *cannot* excrete more urea than this. It is more likely that the patients, being as ill as they are, do not give their kidneys more urea than this to excrete.

The hypobromite method is only approximate; more nitrogen is given off than corresponds to the urea alone, less than to the total nitrogen in the urine. For exact estimations of the urea, the complicated Mörner-Sjöquist process should be used (see Schäfer's 'Text-Book of Physiology,' vol. i.).

4. **Estimation of Uric Acid.**

Hopkins' Potassium Permanganate Method.—To 100 c.c. of urine in a flask add 35 grammes of ammonium chloride. Allow to stand for fifteen minutes after this has dissolved. Insoluble ammonium urate is precipitated. Filter. Wash the precipitate with hot ammonium chloride solution. Open out the

filter-paper, and with hot distilled water wash the precipitate into a 100 c.c. flask. Dilute accurately to 100 c.c. with water. Pour into a beaker, add 20 c.c. strong sulphuric acid, and from a burette drop in $\frac{N}{20}$ permanganate solution made up as follows:

Potassium permanganate .. .. 7·9 grammes
Distilled water .. .. .. 1,000 c.c.
1 c.c. corresponds to 0·00375 gramme of uric acid.

The crimson fluid as it drops in is at first rapidly decolourized; presently the colour disappears more slowly. When the liquid in the beaker retains a distinctly pink colour for fifteen seconds the end reaction is reached. Suppose 8 c.c. of permanganate solution have been added;

∴ in 100 c.c. of urine there are $8 \times 0{\cdot}00375$ grammes uric acid—*i.e.*, 0·03 per cent.

The average total uric acid in twenty-four hours is 0·5 gramme. (*N.B.*—For very accurate estimations, certain 'corrections' have to be made.)

5. **Estimation of Chlorides.**

Volhard's Method.—This consists in adding excess of silver nitrate to precipitate all the chlorides, and determining the excess of silver nitrate with standard solution of potassium sulphocyanide.

Standard Silver Nitrate Solution.

Fused silver nitrate .. .. 29·07 grammes
Distilled water to .. .. 1,000 c.c.
1 c.c. corresponds to 0·01 gramme of sodium chloride.

Standard Potassium Sulphocyanide (KCNS) Solution.

Potassium sulphocyanide .. .. 8·29 grammes
Distilled water to .. .. 1,000 c.c.
2 c.c. corresponds to 1 c.c. of standard silver nitrate solution.

A strong solution of iron alum is also required as indicator, and some pure nitric acid.

To 10 c.c. of urine in a 100 c.c. flask add 5 c.c. of pure nitric acid and excess of standard silver solution, say 25 c.c., measured from a burette. Dilute with water to 100 c.c. Filter off the precipitated silver chloride. Pipette 50 c.c. of the filtrate into a beaker, add 5 c.c. of iron alum solution, and then standard KCNS solution from a burette. The end reaction is the appearance of a permanent red colour, due to formation of sulphocyanide of iron when all the silver has been precipitated.

Suppose 8 c.c. of KCNS solution were thus needed,

2 c.c. of KCNS solution corresponds to 1 c.c. of standard silver solution;

∴ 8 c.c. of KCNS solution corresponds to 4 c.c. of standard silver solution.

∴ in 50 c.c. of the filtrate there were 4 c.c. of silver solution in excess;

∴ in 100 c.c. there were 8 c.c. of silver solution in excess.

But in the 100 c.c. there were 10 c.c. of urine; therefore, of the 25 c.c. of silver solution added to the 10 c.c. of urine, 8 c.c. were in excess.

∴ to precipitate the chlorides in 10 c.c. of urine 25 − 8 c.c. silver solution are required = 17 c.c. But 1 c.c. silver solution corresponds to 0·01 gramme of sodium chloride.

∴ 10 c.c. of urine contain $17 \times 0{\cdot}01$ grammes of sodium chloride;

∴ 100 c.c. of urine contain $17 \times 0{\cdot}01 \times 10$ grammes of sodium chloride = 1·7 per cent.

The method is exceedingly accurate, and the end reaction very definite.

Upon full diet about 15 grammes of sodium chloride are excreted per diem.

Mohr's Method.—To 10 c.c. of urine in a beaker add 100 c.c. of distilled water and 20 drops of saturated potassium chromate solution. Add standard silver nitrate from a burette; a distinct red colour appears when all the chlorides have been precipitated. Suppose 12 c.c. of siver nitrate solution have been added;

∴ in 10 c.c. urine there are $12 \times 0{\cdot}01$ grammes of sodium chloride;

∴ in 100 c.c. urine there are $12 \times 0{\cdot}01 \times 10$ grammes of sodium chloride = 1·2 per cent.

6. Estimation of Phosphates.

The following reagents are needed:

(1) *Standard Uranium Nitrate Solution.*

Uranium nitrate	35·5 grammes
Distilled water to	1,000 c.c.

1 c.c. corresponds to 0·005 gramme of phosphoric acid.

(2) *Sodium Acetate Solution.*

Sodium acetate	10 grammes
Glacial acetic acid	10 c.c.
Distilled water to	100 ,,

(3) *Potassium Ferrocyanide: Saturated Solution.*

To 50 c.c. of urine add 5 c.c. of sodium acetate solution. Warm to 80° C. Add standard uranium nitrate from a burette. The end reaction is the appearance of a distinct brown colour when a drop of the fluid in the beaker is added to a drop of the potassium ferrocyanide upon a white slab.

Suppose 11 c.c. of uranium nitrate have been added; then in 50 c.c. of urine there are $11 \times 0{\cdot}005$ grammes of phosphoric acid;

$\therefore$ in 100 c.c. of urine there are $11 \times 0.005 \times 2$ grammes of phosphoric acid $= 0{\cdot}11$ per cent.

On full diet the average is 3·5 grammes per diem.

7. **Cryoscopy.**—By this is meant the determination of the degree below zero Centigrade to which the freezing-point of the urine is reduced. It has been stated that it is of some value in determining the efficacy of the kidney functions. The process is complicated, and needs special apparatus. Full directions as to use are sold with the latter, so that they need not be given here.

8. **Estimation of the Purin Bases.**—There are many substances allied to uric acid—xanthin, hypoxanthin, guanin, and so forth—which are known collectively by the term 'purin bases.' There is a special apparatus for estimating these—the purinometer. It is not difficult to use. Full instructions are sold along with the apparatus.

CHAPTER II.

EXAMINATION OF THE BLOOD.

Blood is best obtained from the lobule of the patient's ear, which, after cleaning with soap and water and thorough drying, should be held firmly at its base between the finger and thumb of the left hand, and given one *rapid* deep puncture with the needle. An ordinary straight surgical needle answers admirably; a straight Hagedorn's needle is best of all. The blood should well up freely, and squeezing should be avoided, lest lymph be expressed with it.

Enumeration of the Red and White Corpuscles:—

The best instrument to use is the **Thoma-Zeiss Hæmocytometer**; this consists of: (1) counting chamber; (2) specially ground cover-glass; (3) red corpuscle pipette; (4) white corpuscle pipette.

The counting chamber (Fig. 28) is made of an outer flat ring of glass, R, separated by a circular trench, T, from a central platform, P, firmly cemented to a glass slide. The outer ring is $\frac{1}{10}$ mm. higher than the platform, so that the cover-glass, resting flat upon the former, is separated from the latter by $\frac{1}{10}$ mm. The centre of the platform is ruled into minute squares

(Fig. 29), each side of which measures $\frac{1}{20}$ mm.; the cubic space over each small square, between the platform below and the cover-glass above, is therefore

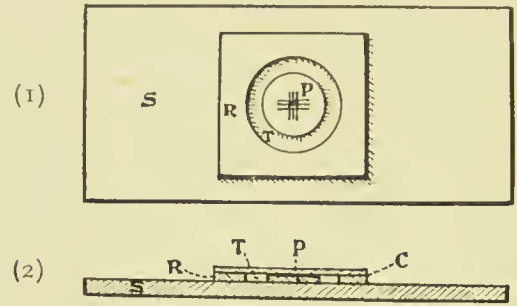

FIG. 28.—Blood-counting Chamber.
T=trench, P=platform, R=ring, S=slide, C=cover-glass.
(1) Surface view. (2) Side view in sectra.

$\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cub. mm. As a rule the small squares are arranged in sets of sixteen by double rulings at

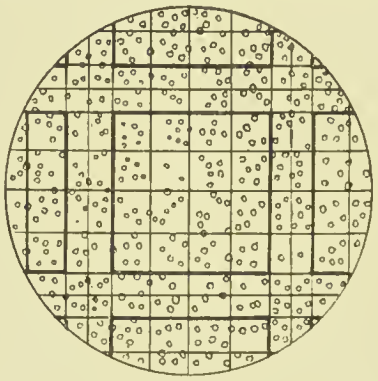

FIG. 29.—Hæmocytometer Squares and Blood Corpuscles as seen under Fairly Low Power.

intervals. Such sets of sixteen small squares are termed 'big squares.'

The stem of the red corpuscle pipette is graduated into tenths up to a mark 1; the bulb contains a glass

bead to facilitate mixing; above the bulb is a mark 101 (Fig. 30 A).

The white corpuscle pipette is similar, but the bore is wider, the bulb smaller, and the upper graduation is 11 instead of 101 (Fig. 30, B).

The diluting fluids required are the following:

Hayem's Fluid, for dilution in counting red corpuscles:

Mercury perchloride	..	0·25 gramme
Sodium chloride	..	0·5 ,,
Sodium sulphate	..	5·0 grammes
Distilled water	..	100·0 c.c.

Or one may use a 0·8 per cent. solution of common salt, which is isotonic with human blood, and therefore neither lakes nor crenates the corpuscles.

Thoma's Fluid, for dilution in counting white corpuscles:

Acetic acid (B.P.)	.. ..	1·0 c c.
Gentian violet solution	..	0·5 ,,
Distilled water	.. ..	100·0 ,,

In this fluid the red corpuscles are laked; their ghosts alone are seen. The white corpuscles stand boldly out, their nuclei being just tinged with the stain.

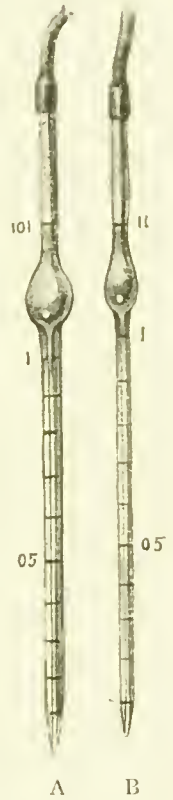

FIG. 30.—Hæmocytometer Pipettes.

A, for diluting blood to count the red cells; B, for diluting blood to count the white cells.

It is convenient to reserve for blood-counts, either upon a small tray or in a case, five wide-necked stoppered bottles. In the first keep Hayem's fluid, in the second Thoma's fluid, in the third, fourth, and fifth distilled water, absolute

alcohol, and ether respectively. The three latter are for cleaning the pipettes. Immediately a pipette is done with blow out the contents; draw up distilled water from bottle 3 into the bulb, and blow it out again; repeat this three times. Now draw up absolute alcohol from bottle 4 in a similar way three times. Finally, draw up ether from bottle 5 three times; after blowing out the ether the last time, draw air through the bulb. The water cleans the pipette, the alcohol dehydrates it, the ether replaces the alcohol, itself rapidly evaporates, and leaves the apparatus clean and dry, ready for use. Done at once, the cleaning takes a minute; left till next day, perfect cleaning may be difficult, or, worse still, the pipette may be completely clogged.

To Count the Red Corpuscles.—Prick the ear, wipe away the first drop of blood, wait till a second the size of a hemp-seed has collected, immerse the end of the pipette in it, and draw blood up to the mark 0·5. Quickly wipe the end of the pipette, plunge it into bottle 1, draw up Hayem's fluid to the mark 101; pinch the rubber tube, hold the pipette horizontally, and rapidly roll the bulb to and fro to mix the contents thoroughly. The blood dilution is 1 in 200. The upper mark is 101, and not 100, because the Hayem's fluid in the stem up to the point 1 takes no part in the mixture. Get rid of it in the first drop that is blown from the pipette, and then put part of the next drop on to the central platform of the counting chamber. With a little practice it is easy to judge the exact amount required for this. Apply the cover-glass, and press it down. If the fluid runs off the platform into the

trench, too big a drop has been taken. Part of a drop only is required; when covered, it should just extend to the margin of the platform, but not over the edge. If it do so, or if a bubble of air get in, clean the counting chamber, and take a fresh sample. Enumerate the corpuscles under a $\frac{1}{6}$-inch objective, counting the number in each of the sixteen small squares which make up one big square; and count five sets of sixteen small squares.* The variations are considerable. The following is an example of a count in one big square:

3	8	10	5	} Total 100.
7	7	9	1	
6	1	13	5	
10	6	5	4	

Corpuscles often overlap dividing lines. Such are apt to be counted twice, unless a rule be made to count into a square those which overlap its top and right-hand, but not those over the bottom and left-hand, sides (Fig. 31).

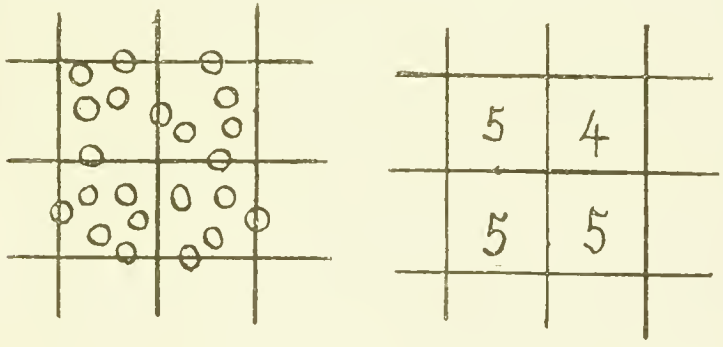

FIG. 31.—Counting Red Corpuscles which overlap the Dividing Lines.

* The reason for counting the red cells in five big squares rather than in more is that the subsequent calculations are thereby greatly simplified (see p. 64).

Having counted five big squares, the figures might be:

$$\left.\begin{matrix} 100 & 92 & 110 \\ 96 & 102 & \end{matrix}\right\} \text{ Total } 500.$$

The average is in this case 100 to the big square, or $\frac{100}{16}$ to the small square. But the volume of fluid over each small square is $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cub. mm. (p. 60).

$\therefore$ in $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cub. mm. of 200 times diluted blood there were $\frac{100}{16}$ red corpuscles.

$\therefore$ in 1 cub. mm. of 200 times diluted blood there were $\frac{100}{16} \times \frac{20 \times 20 \times 10}{1}$ red corpuscles.

$\therefore$ in 1 cub. mm. of original blood there were $\frac{100}{16} \times \frac{20 \times 20 \times 10}{1} \times \frac{200}{1}$ red corpuscles.

= 5,000,000 red corpuscles per cub. mm.

It will thus be seen that, with a dilution of 1 in 200, normal blood containing 5,000,000 red corpuscles per cub. mm. gives 100 to the big square. Hence, the average number in a big square, the dilution being 1 in 200, gives the percentage of red corpuscles without further calculation; and the number in five big squares, with the addition of ...0,000, is the number of red corpuscles per cub. mm. of original blood.

Importance of Counting the Red Corpuscles.—Less can be learned from the number of red corpuscles alone than from the number of red corpuscles and the amount of hæmoglobin at the same time (p. 70). A patient may have a normal number of red corpuscles and yet may be very anæmic from deficiency of hæmoglobin.

Apart from the hæmoglobin, however, a count of the red corpuscles may be by itself valuable in the following ways:

(1) In congenital heart disease (morbus cæruleus) the red corpuscles are frequently much increased—in some cases to 7,000,000 per cub. mm.; in a few to double this. A similar but less marked increase occurs in those cyanosed from other causes, such as fibroid lung or mitral stenosis.

(2) Those who live at high altitudes usually have more than 5,000,000 per cub. mm.

(3) As an anæmic patient improves, the red corpuscles increase more rapidly than does the hæmoglobin. Hence, enumeration of the red corpuscles from week to week affords a valuable means of gauging the effect of treatment.

(4) There is a peculiar disease in which the main characteristics are—(*a*) considerable enlargement of the spleen, (*b*) great increase in the number of red corpuscles (polycythæmia), up to 10,000,000 per cub. mm. or more. It is called splenomegalic polycythæmia.

To Count the White Corpuscles.—Proceed in an exactly similar manner, but with the other pipette. The bore being larger, a bigger drop of blood is required. Care is needed to prevent the fluid running out. Fill to the mark 0·5 with blood; dilute with Thoma's fluid to the mark 11. The dilution is then 1 in 20. Use the same counting chamber, with the same precautions. In normal blood, diluted twenty times, there are seldom more than three leucocytes in each *large* square, and often none at all in some. Count sixteen large squares; suppose there are a total of twenty

leucocytes, the average in one large square would be $\frac{20}{16}$, and in one small square $\frac{20}{16 \times 16}$.

$\therefore$ in $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cub. mm. of twenty times diluted blood there are $\frac{20}{16 \times 16}$ leucocytes.

$\therefore$ in 1 cub. mm. of twenty times diluted blood there are $\frac{20}{16 \times 16} \times \frac{20 \times 20 \times 10}{1}$ leucocytes.

$\therefore$ in 1 cub. mm. of original blood there are $\frac{20}{16 \times 16} \times \frac{20 \times 20 \times 10}{1} \times \frac{20}{1}$ leucocytes.

$= 6{,}250$ leucocytes per cub. mm.

The normal number of leucocytes is usually given as 5,000 per cub. mm.; but it must be remembered that there are daily variations in every person, and that it is the rule rather than the exception to find as few as 4,000 and as many as 12,000 in the same person at different times on the same day. Unless the numbers are above 15,000 or below 4,000 they cannot be called abnormal.

Importance of Counting the White Corpuscles.—Leucocytosis—*i.e.*, an increase in the total number of leucocytes to more than 18,000 per cubic mm.—often accompanies deep-seated suppuration, and may afford valuable help in diagnosis. Absence of leucocytosis does not exclude suppuration. A leucocytosis which increases at successive counts is strongly in favour of progressive pus formation, and therefore of operation —for instance, in case of appendicitis. Suppuration often causes a leucocytosis up to 20,000 or 25,000;

it rarely reaches 60,000, and almost never exceeds this.

In lobar pneumonia there is often leucocytosis up to 30,000 or more.

In typhoid fever and malaria without complication there is no leucocytosis, but rather a leucopenia—*i.e.*, an actual diminution in the leucocytes below 4,000 per cub. mm. In many cases in which obscure pyrexia suggested typhoid fever or malaria, leucocytosis to 20,000 has led to the discovery of deep-seated pus—for example, in a pyosalpinx or hepatic or appendicular abscess.

In the diagnosis of the leuchæmias, lymphatic and spleno-medullary, from Hodgkin's disease and splenic anæmia, a leucocyte count is indispensable. In the two last there is no leucocytosis. In lymphatic leuchæmia the leucocytes rise to as many as 150,000 per cub. mm., whilst in the spleno-medullary form 200,000 are often found, and even 600,000.

Estimation of the Hæmoglobin.

Haldane's Hæmoglobinometer or Gower's Hæmoglobinometer.—The older forms of hæmoglobinometer need not be described. Where coal-gas can be obtained no instrument is better than Haldane's. Where there is no supply of coal-gas Gower's may be used. The two can be combined. Haldane's apparatus (Fig. 32) consists of (1) a sealed tube A,* containing a standard* solution of carboxyhæmoglobin; (2) a graduated tube B, for diluting the

* It is standardized, not empirically, but by actual gas analysis of the blood from which it was prepared.

blood in ; (3) an indiarubber stand C for these tubes ; (4) a pipette D for measuring the blood ; (5) a brass U-tube E for introducing the coal-gas into B ; (6) a drop-bottle F for distilled water. The method is as follows : Drop distilled water into the tube B up to the mark 10. Draw blood into the pipette up to the point indicated on it. Expel it into the

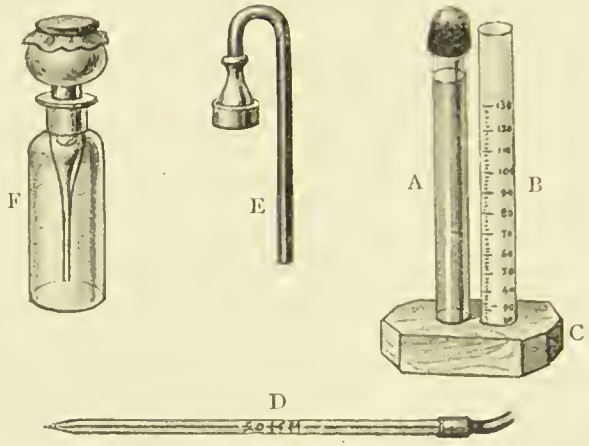

FIG. 32.—Haldane's Hæmoglobinometer.

distilled water in B without immersing the tip of the pipette. Shake the tube B. The blood is laked at once. Now wash out the pipette as directed on p. 62. Fit the short end of the brass U-tube on to an unlighted gas-burner, and push the tube B up over the longer limb until the latter nearly touches the laked blood (Fig. 33). Turn on the gas, and slowly draw away the tube B. The air in its upper part is replaced by coal-gas. On shaking up the laked blood with the gas, the carbon monoxide of the latter converts the hæmoglobin into cherry-red carboxy-

hæmoglobin. Repeat the introduction of coal-gas if necessary. Now add distilled water drop by drop to the tube B, mixing it up each time, until the tint of the fluid is identical with that of the standard carboxyhæmoglobin in tube A. The graduation now corresponding to the upper level of the fluid in tube B is the percentage of hæmoglobin in the patient's blood, that of normal blood being 100. It is well to make two readings, the first when the tint is just perceptibly darker than the standard, the other when just paler, and to take the mean of the two. With practice the error becomes very small.

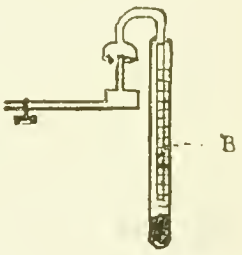

FIG. 33.

Gower's apparatus is very similar in its various parts, but the standard colour-tube contains carmine solution instead of carboxyhæmoglobin, and the laked blood is immediately diluted drop by drop until the tint of mixed oxy- and reduced hæmoglobin is as nearly as possible identical with that of the carmine. The tints can only be made to agree in good daylight, whereas in Haldane's method the solutions are both the same colouring matter, carboxyhæmoglobin, and therefore can be compared in any light, artificial or natural. Carboxyhæmoglobin, moreover, is a very stable compound, and the blood can be kept for examination at any time. Oxyhæmoglobin rapidly changes in shed blood, so that, with Gower's method, comparison must be made at once.

In country districts it is well to have both a carmine and a carboxyhæmoglobin standard colour-tube for

the same apparatus. In the event of coal-gas being unprocurable the former could be used; in all other cases the latter.

Tollquist's Hæmoglobinometer.—This is not strictly accurate, but it is very simple, and for many purposes the readings are near enough. It consists of a book containing papers of various tints of red and some white blotting-paper. A drop of the patient's blood is received upon a piece of the latter, and as soon as it is dry its colour is compared with those of the tinted papers until one to match it is found. The percentage of hæmoglobin is thus found in a couple of minutes.

Importance of Estimating the Hæmoglobin.—The severity of most anæmias is better gauged by the amount of hæmoglobin than by the number of red corpuscles. In chlorotic girls the latter may be but little below normal, and yet the hæmoglobin may be very much reduced.

The **colour index** of the blood is the ratio of hæmoglobin to red corpuscles. Three types of anæmia are based upon it. In healthy blood the colour index is 1; in anæmia directly following hæmorrhage it is still 1; in the chlorotic type it is less than 1; in the pernicious type greater than 1. These characteristics are represented by arbitrary examples in the following table:

	Percentage of Red Corpuscles.	Percentage of Hæmoglobin.	Colour Index.
Healthy person	100	100	$\frac{100}{100}$, or 1.
Anæmia from hæmorrhage	80	80	$\frac{80}{80}$, or 1.
Chlorotic type	60	30	$\frac{30}{60}$, or 0·5.
Pernicious type	20	30	$\frac{30}{20}$, or 1·5.

Thus, in anæmia immediately following hæmorrhage red corpuscles and hæmoglobin are equally diminished; in chlorotic types the hæmoglobin is diminished more than are the red corpuscles; in pernicious anæmia the red corpuscles are more diminished than is the hæmoglobin.

Most anæmias are of the chlorotic type. For example, chlorosis itself; tuberculous and cancerous cachexias; infective endocarditis and other toxæmias; the later stages of Hodgkin's disease, the leuchæmias, splenic anæmia.

To the pernicious type belong pernicious anæmia and some cases of ankylostomiasis. Without estimations of both red corpuscles and hæmoglobin, and a calculation of the colour index, some cases of infective endocarditis or carcinoma of the stomach may be wrongly diagnosed as pernicious anæmia.

Fig. 34.—Bottle for staining Blood-films in.

The Preparation and Examination of Blood-films.

The least wasteful way of storing the various stains is to use cylindrical glass jars (Fig. 34), $3\frac{3}{4}$ inches high, $1\frac{1}{2}$ inches diameter, with ground stoppers. Films can be immersed bodily in these, and the same stain used again and again.

Films may be made either upon microscope slides or upon cover-glasses. If the latter are used, they should be the thinnest possible (No. 1); others are too thick for use with the oil-immersion lens. In

almost all cases, however, it is more convenient to make the film upon a slide. When it is finished and stained, there is no need to cover it, nor to use Canada balsam; cedar-wood oil may be put directly on to the dried and stained film, and the latter can be examined with the oil-immersion lens without any other covering at all. The same applies to films of pus to be examined for bacteria. The slides may even be preserved for future reference uncovered; but if they are to be kept for any length of time, it is better to cover them in the usual way to prevent their getting scratched.

To make a film, a good plan is the following: Cut a cigarette-paper at right angles to its length into strips the same width as the slips. Prick the ear; wipe away the first drop of blood; when the second is as large as a big pin's head, smear the ungummed end of a strip over its summit. Holding the other end, lay the smeared surface upon a cover-glass, and draw it steadily along (Fig. 35). The thickness of the film can be varied by altering the angle at which the paper is held to the glass. The blood should dry almost as quickly as it is spread. It is too thick if it stand in slow-drying pools. Use a fresh strip for each film; never smear the cover-glass over twice.

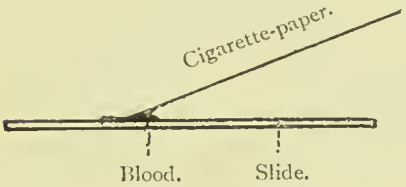

FIG. 35.—Cigarette-paper Method of making Blood-film.

Another plan which is very simple and good, but which is more easily demonstrated than described, is to receive a small drop of blood on the surface of one slide near its end, and then, laying the edge of another

slide across the first through the middle of the blood-drop, push the latter steadily along the surface of the first slide. A perfectly even film is thus obtained.

Unless carefully fixed, the corpuscles wash off the glass in the process of staining.

Methods of Fixing Films.—Four of the chief methods of fixing films are as follows :

1. **In Absolute Alcohol and Ether.**—This requires no special apparatus. Fill three-quarters of one of the glass jars with absolute alcohol and sulphuric ether, equal parts. Drop in the films when dry, and leave them till next day. An hour is said to be enough, but twenty-four are better.

2. **By Heat.**—A hot-air oven with a good gas regulator is required. Fix for two hours at a temperature of 120° C., or for half an hour at 140° C. For certain stains fixing by heat is essential, but it is inconvenient in private practice.

3. **By Jenner's Method** (see below), which fixes and stains at the same time.

4. **By Formalin.**—Immerse the films in a 1 per cent. solution of formalin for one minute.

Methods of Staining Films.—Many different ways of staining blood-films have been recommended, but it will serve no useful purpose to describe in detail any but those which are widely acknowledged to be the simplest and best—namely :

For ordinary purposes	1. Jenner's method. 2. The hæmatoxylin and eosine method.
For malaria and other hæmatozoa	3. Leishmann's method.

1. **Jenner's Method.**—This has a paramount advantage, in that it fixes and stains at the same time, and therefore is very rapid. The films may be stained and ready for examination ten minutes after the patient's ear has been pricked. The method is a little uncertain, but practice makes it less and less so. There is considerable variation in the efficacy of different bottles of the stain. The main point, however, upon which success or otherwise depends, is the condition of the wash-water.

Formula for Jenner's Stain.

Crystalline methylene-blue eosine ..	0·5 gramme
Methyl alcohol	100 c.c.

It is well to keep the wash-water in a special jar, and to use the same again and again. It is essential that distilled water be used; but even distilled water may be too alkaline, from the presence of ammonia, in which case too much of the blue stain will be removed. It is possible to correct this by accurate titration with very dilute acetic acid; but a simpler plan is to pour a few drops of the Jenner's stain into the jar of wash-water, so as to saturate the latter with blue, and then, dirty though it may appear to be, to keep this carefully for the washing of future films. To stain, drop the film, as soon as it has dried after making, into the Jenner's stain for five minutes; then lift it out, allow as much stain as possible to run off, and transfer the film to the wash-water; move it gently to and fro for about half a minute; when it looks quite pink, remove it from the wash-water; dry with filter-paper and examine.

The red corpuscles are bright pink; their nuclei, if present, blue. The large lymphocytes have pale blue nuclei and paler blue clear protoplasm. Small lymphocytes have deep blue nuclei and a rim of pale blue protoplasm. Polymorphonuclear cells have blue nuclei, and pink protoplasm containing fine pink granules. Eosinophile corpuscles have blue nuclei and pink protoplasm full of very big red granules. Myelocytes have dark blue nuclei and pale blue protoplasm, the latter containing either blue streaks and granules or, in the case of eosinophile myelocytes, big red granules. The granules in any basophile cells that may be present are deep violet. Bacteria, malaria, or filaria parasites are pale blue if stained at all.

2. **Delafield's Hæmatoxylin and Aqueous Eosine Method.**—The disadvantage of this method is that it is slow; its advantages are—first, that it is very certain; secondly, that no distilled water is necessary; for the washing not only may be, but must be, done with ordinary tap water.

Delafield's Hæmatoxylin.—To 400 c.c. of a saturated aqueous solution of ammonia alum add 4 grammes of hæmatoxylin dissolved in 25 c.c. of absolute alcohol. Leave the solution exposed to the light and air in an unstoppered bottle for three or four days; filter, and add to the filtrate 100 c.c. of glycerine and 100 c.c. of methyl alcohol. Allow the solution to stand in the light until it is a dark colour, refilter, and preserve in a stoppered bottle.

Aqueous Eosine Solution.

Eosine (aqueous)	..	..	..	1 gramme
Distilled water	..	..	..	100 c.c.

The films should be fixed for twenty-four hours in absolute alcohol and ether (p. 73); the procedure is then as follows:

Wash with tap water to remove alcohol and ether; blot with filter-paper; immerse in hæmatoxylin for five minutes; wash thoroughly in running tap water: the slight alkalinity of the latter serves to 'blue' the hæmatoxylin; blot; immerse in eosine for thirty seconds; wash again in running tap water; dry with filter-paper, and examine.

The red corpuscles are stained pink; their nuclei, if present, blue. The small and large lymphocytes have deep purplish-blue nuclei and pale protoplasm of the same colour. Myelocytes stain similarly to large lymphocytes, but are distinguished by the granules in their protoplasm. Polymorphonuclear cells have bluish-purple nuclei and bright pink protoplasm, the latter showing fine granules far less distinctly than with Jenner's stain. Eosinophile cells have bluish purple nuclei and bright pink protoplasm crowded with large pink granules. Basophile cells and hæmatozoa do not stain well by this method.

3. **Leishmann's Method.**—This is the stain, *par excellence*, for malaria parasites. It is also a very good way of staining ordinary blood-films; it is similar to Jenner's, and, like the latter, fixes and stains at the same time. The stain consists of a modified eosinate of methylene blue dissolved in methyl alcohol; it is the latter which fixes the films. The formula is—

Eosinate of methylene blue (Grübler)	0·15 gramme
Methyl alcohol (Merck)	100 c.c.

The dye dissolves with difficulty in the methyl alcohol. A good plan is to put the eosinate into a mortar and pour on 20 c.c. of the alcohol at a time, rubbing well with the pestle till the whole 100 c.c. has been used. Place the slide, film uppermost, upon a perfectly horizontal table; filter just enough of the stain on to the film to cover the latter evenly; leave for one minute, covering with a large watch-glass to prevent evaporation and precipitation of the stain. Now pour on distilled water drop by drop till as much has been added as there was stain originally; leave the slide for another five minutes, and then wash thoroughly with distilled water, to which some Leishmann's stain has been added as described for Jenner's stain (p. 74); blot; dry, and examine.

The red corpuscles are bright pink; the nuclei of the leucocytes are dark reddish-purple; malaria parasites are bright blue with almost black granules.

It is probable that anyone who has tried the above methods will not feel any need for others. The following have all at one time or other been much in vogue, but they are now less in fashion.

4. **Methylene Blue and Eosine.**—(*a*) Alcoholic solutions for films fixed by heat:

Alcoholic Eosine.

Eosine	0·5 gramme
Distilled water	30·0 c.c.
Absolute alcohol	70·0 ,,

Alcoholic Methylene Blue.

Methylene blue	1·5 grammes
Rectified spirit	100·0 c.c.

Allow to stand for twenty-four hours, shaking vigorously from time to time. Filter. Stain in the eosine first, for one minute; wash in distilled water; blot; stain in methylene blue for five minutes; wash in distilled water, and dry.

(*b*) Aqueous solutions, for films fixed in alcohol and ether:

Watery Eosine (see p. 75).

Watery Methylene.

Methylene blue	1·5 grammes
Distilled water	100·0 c.c.

Shake up daily for two weeks. Filter.

Stain the film in eosine for one minute; wash in distilled water; blot; stain in methylene blue for five minutes; wash in distilled water, and dry.

These two methods are uncertain, though when successful they are pretty. If the methylene blue stains faintly, try warming it gently.

5. **Ehrlich's Triple Stain**:

Saturated solution of orange G.	13·14 c.c.
Saturated solution of acid fuchsine ..	6·7 ,,
Distilled water	15·0 ,,
Absolute alcohol	15·0 ,,
Saturated solution of methyl green ..	12·5 ,,
Absolute alcohol	10·0 ,,
Glycerine	10·0 ,,

Measure off the ingredients in the order named. The mixture may be used at once; but to make each of the saturated solutions it contains requires several days. The stain keeps well, but whereas one batch may stain excellently, the next may fail entirely.

Films must be fixed by heat. Immerse in the stain for one to five minutes; the time varies with different

bottles of stain and with the length of time the films have been fixed by heating. Wash with *distilled* water, and dry.

6. **Romanowski's Method for Malarial Parasites.**—This is inferior to Leishmann's stain in that the films have to be fixed, either by alcohol and ether or by heat, before staining. The stain consists of a mixture of 1 part of saturated aqueous methylene blue and 2 parts of 1 per cent. aqueous eosine. The slides, when fixed, are immersed in this for two hours, washed in distilled water, and dried.

Examination of the Film.—This should be made with $\frac{1}{12}$-inch oil-immersion lens, a substage condenser, and a mechanical stage. Select that part of the film in which the corpuscles do not overlap. A differential leucocyte count may be made at the same time. Look for the following points:

Changes in the Red Corpuscles.—In a well-fixed film of normal blood these are smooth in outline, quite round, of approximately equal sizes, and stained rather less at the centre than at the periphery. If the cells are crenated or run together into batches so as to compress one another into polygonal shapes (Fig. 37), the fault is in the film-making.

In severe anæmias red corpuscles may vary—

1. **In Size.**—A normal red corpuscle, 7 μ in diameter, is termed a **normocyte**; a smaller is a **microcyte**; a larger a **megalocyte** (Figs. 36 and 38).

2. **In Shape.**—Instead of round, they may be pear-shaped, oval, fiddle-shaped, or ovoid; such corpuscles are termed **poikilocytes**, and the condition **poikilocytosis** (Fig. 39).

3. **In Staining Capacity.**—A normocyte should stain throughout. When hæmoglobin is very deficient, the corpuscles may resemble quoits, a thin rim of red surrounding a colourless interior. In severe anæmias, and particularly in pernicious anæmia, some of the red cells lose their affinity for acid dyes, and stain bluish-red or violet instead of pink. The condition is termed **polychromatophilia** (Fig. 41). It has little import except that it is never found in normal blood.

4. **In the Presence of Nuclei.**—Nucleated red corpuscles have a deep blue nucleus and a rim of pink protoplasm, which readily distinguishes them from the lymphocytes, in which both nuclei and protoplasm are blue. A nucleated normal-sized red corpuscle is termed a **normoblast**; a nucleated small corpuscle a **microblast**; a large one a **megaloblast**. Occasionally more than one nucleus is present in a still larger cell, termed a **gigantoblast** (Fig. 40).

Importance of the Changes in the Red Corpuscles.—Poikilocytosis, alterations in size, and presence of nucleated red corpuscles, may occur in *any* severe anæmia. Though seen most typically in pernicious anæmia, these changes are not pathognomonic. If there is a *considerable preponderance of megalocytes*, together with poikilocytosis, and no leucocytosis, there is great likelihood that the condition is pernicious anæmia.

A high proportion of nucleated red corpuscles indicates very severe anæmia, and a less good prognosis; conversely, if the proportion of nucleated forms diminishes, the patient is improving.

The Differential Leucocyte Count.—A mechan-

DESCRIPTION OF PLATE I

FIG. 36.—Normocytes.
,, 37.—Badly Fixed and Crenated Red Cells.
,, 38.—Megalocytes and Microcytes.
,, 39.—Poikilocytes.
,, 40.—Nucleated Red Corpuscles.
,, 41.—Polychromatic Red Corpuscles.
,, 42.—Small Lymphocyte.
,, 43.—Large (hyaline) Lymphocyte.
,, 44.—Polymorphonuclear Leucocyte.
,, 45.—Coarsely Granular Eosinophile Leucocyte.
,, 46.—Myelocyte.
,, 47.—Eosinophile Myelocyte.
,, 48.—Basophile Leucocyte.
,, 49.—Malaria Parasite: Ring Form.
,, 50.—Filaria Embryo and Red Cells.
,, 51.—Malaria Parasite: Crescent Form.
,, 52.—Spirilla of Relapsing Fever and Red Cells.

Plate I.

Fig.36.

Fig.37.

Fig.38.

Fig.39.

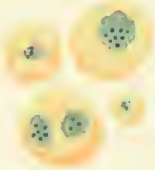

Fig.40.

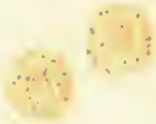

Fig.41.

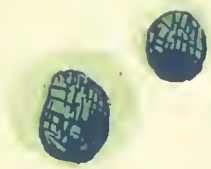

Fig.42.

Fig.43.

Fig.44

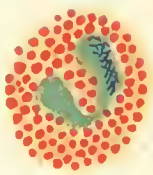

Fig.45.

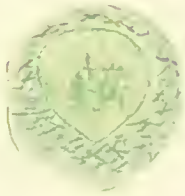

Fig.46.

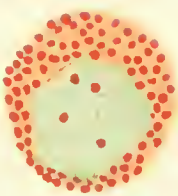

Fig.47.

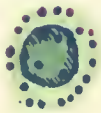

Fig.48.

Fig.49.

Fig.50.

Fig.51.

Fig.52.

ical stage is essential. Omit the thicker parts of the film; in the remainder examine the leucocytes consecutively, allotting each to its appropriate class. Begin at one end of the film, say the top right-hand corner, and screw it continuously past the eye until the left edge of the thin part is reached; then, watching through the microscope, screw it down one field's breadth, and then count the leucocytes continuously back again until the right edge is reached; turn the film down another field's breadth, and count it transversely across to the left edge again; and so on, backwards and forwards, until the required number of corpuscles, say 250, has been enumerated. In normal blood these will fall into four main groups, to which various names have been given. They are—

1. **Small Lymphocytes** (Fig. 42). — These are slightly larger than red corpuscles, variable in size, characterized by a comparatively large, deep-staining spherical nucleus and a rim of protoplasm which is sometimes very narrow, sometimes considerable. These constitute from 20 to 30 per cent. of leucocytes in health.

2. **Large Lymphocytes** (Fig. 43).—Also termed 'hyaline'; these are much larger cells, with notched or kidney-shaped nucleus, and much pale-staining clear protoplasm. These constitute from 2 to 10 per cent. in health. Some cells are intermediate between typical small and typical large lymphocytes, classed with the former by some, by others with the latter. Most class all such intermediate cells with the small lymphocytes, reserving the term 'large lymphocytes' for the typical large cells only.

3. **Polymorphonuclear Cells** (Fig. 44).—These are somewhat smaller than large lymphocytes; usually circular, with a well-defined nucleus variously lobed and twisted. The name of this cell is derived from this characteristic of the nucleus, which may be horseshoe-shaped, or twisted like the letters Z, S, E. Other names used are 'neutrophile' and 'finely granular oxyphile.' The protoplasm is abundant, and shows minute red granules; these cannot be mistaken for the coarse granules of the next variety when once the latter have been seen. The polymorphonuclear cells constitute about 65 per cent. of all leucocytes in health.

4. **Coarsely Granular Eosinophile Cells, or 'Eosinophiles'** (Fig. 45).—These are slightly larger than the polymorphonuclear cells, with a similar twisted nucleus, and protoplasm which is crowded with unmistakable coarse red granules. They vary from 0·5 to 5 per cent. in health.

Two other forms of leucocytes are found under certain pathological conditions, namely:

5. **Myelocytes** (Fig. 46).—These are large cells with round or oval pale-staining nuclei, and much protoplasm, in which there are many granules and streaks, by which these cells are distinguished from large lymphocytes whose protoplasm is hyaline. In certain myelocytes the granules stain bright red. These **eosinophile myelocytes** (Fig. 47) might be mistaken for coarsely granular eosinophile cells were it not that the nuclei of the latter are lobed, whilst that of each myelocyte is round.

6. **Basophile Cells** (Fig. 48).—These are about the size of small lymphocytes, which they resemble, except

in that their protoplasm contains half a dozen or more very big granules, which are stained deep violet by Jenner's stain..

Importance of the Differential Leucocyte Count.—So variable are the proportions of the white corpuscles in health that too much stress must not be laid upon comparatively slight increases in any particular variety.

The Small Lymphocytes are increased:

Up to 35 per cent. or more in many healthy infants; up to 40, 50, or 60 per cent. in children suffering from congenital syphilis, whooping-cough, and many non-suppurative infantile complaints.

In typhoid fever, especially in mild attacks; in cases of obscure pyrexia, without leucocytosis, an increase of small lymphocytes up to 35 or 40 per cent. favours a diagnosis of enterica, and in the tropics may be a valuable means of excluding malaria.

In lymphatic leuchæmia, in which there is leucocytosis to 50,000 or 150,000 or more, the small lymphocytes often reach as high as 95 per cent., a point which is pathognomonic of the disease.

In many debilitating diseases in adults, such as syphilitic, malignant, or tuberculous cachexia, cirrhosis of the liver, and so forth, provided there be no associated abscess formation, the differential leucocyte count very often shows considerable relative increase of small lymphocytes and diminution of polymorphonuclear cells; no great clinical importance can be attached to the fact, except in excluding suppuration.

The Large Lymphocytes are increased:

In malaria in which the total number of leucocytes is, as a rule, under 5,000 per cub. mm., and the large

lymphocytes may be 15 to 30 per cent.; this affords a valuable means of diagnosis between malaria and enterica, and between malaria and hepatic abscess. The relative increase in large lymphocytes does not disappear immediately on giving quinine, as do the malaria parasites.

The Polymorphonuclear Cells are increased:

In lobar pneumonia and in cases of deep-seated suppuration, such as empyema, appendicular abscess, pyosalpinx. Leucocytosis and a rise of polymorphonuclears to over 80 per cent. may often help in the diagnosis of deep-seated pus.

The Coarsely Granular Eosinophile Cells are increased:

In many cases of true asthma, when they may reach anything between 6 and 60 per cent.

In certain parasitic affections, notably in ankylostomiasis; in *Bilharzia hæmatobia;* in trichinosis; in filariasis; and in patients suffering from tape-worms. The eosinophilia may be slight—up to 10 per cent., for example. It is often between 10 per cent. and 20 per cent., and occasionally reaches 50 per cent. Nothing under 5 per cent. can be termed eosinophilia. There is no eosinophilia with oxyuris vermicularis or trichocephalus dispar; nor is there any with the parasitic skin diseases—tinea tonsurans and so forth.

In certain skin diseases, particularly true pemphigus and hydroa herpetiformis, where from 6 per cent. to 20 per cent. may be found. In other skin diseases, such as psoriasis or eczema, the majority of patients show no eosinophilia, though cases with increase to 5 per cent. or more occur occasionally.

The Myelocytes are present in numbers in one disease only—namely, spleno-medullary leuchæmia, where they may constitute 45 per cent. of all the white corpuscles. They are not, however, distinctive of this condition; 1 or 2 per cent. may be found in splenic anæmia, Hodgkin's disease, pernicious anæmia, or lymphatic leuchæmia.

The Basophile Cells are not pathognomonic of any disease, though they are practically absent in health. They are most numerous in spleno-medullary leuchæmia, reaching to 2 to 5 per cent. Smaller numbers are found in other anæmias, but what importance attaches to this is not yet known.

The following are some specimens of characteristic differential leucocyte counts:

S. = small lymphocytes; L. = large lymphocytes; P. = polymorphonuclear cells; E. = coarsely granular eosinophil cells; M. = myelocytes; B. = basophile cells.

Normal Adult Blood.

	Per Cent.
S.	25
L.	8
P.	65
E.	2
M.	absent
B.	absent

Normal Child's Blood.

	Per Cent.
S.	38
L.	8
P.	53
E.	1
M.	absent
B.	absent

Spleno-medullary Leuchæmia.

	Per Cent.
S.	12
L.	1
P.	37
E.	2
M.	45
B.	3

Lymphatic Leuchæmia.

	Per Cent.
S.	91
L.	2·7
P.	6
E.	often absent
M.	0·2
B.	0·1

Malaria.

	Per Cent.
S.	20
L.	18
P.	60
E.	2
M.	absent
B.	absent

Eosinophilia.

	Per Cent.
S.	25
L.	8
P.	53
E.	14
M.	absent
B.	absent

Abscess Formation.

	Per Cent.
S.	12
L.	2·5
P.	85
E.	0·5
M.	absent
B.	absent

Various Cachexias without Pus Formation.

	Per Cent.
S.	40
L.	6·2
P.	53
E.	0·5
M.	absent
B.	0·3

It should be borne in mind that, apart from malaria and other parasitic conditions, the only diseases in which the blood examination affords pathognomonic evidence of the diagnosis are—

Spleno-medullary leuchæmia.
Lymphatic leuchæmia.
Pernicious anæmia.

In many other conditions a blood examination has to be made in order to exclude one or other of the above. In Hodgkin's disease, for example, the evidence afforded by the blood is negative. One cannot diagnose Hodgkin's disease by examining the blood; but having examined it and found a simple anæmia, leuchæmia is excluded, and Hodgkin's disease remains as a possibility.

The following notes on the main features of the blood in different conditions may be useful.

Spleno-medullary Leuchæmia. — Hæmoglobin

index 1 or less; leucocytosis, possibly to 400,000; myelocytes up to 45 per cent.

Lymphatic Leuchæmia.—Hæmoglobin index 1 or less; leucocytosis up to 150,000 or more; small lymphocytes may be 95 per cent.

Hodgkin's Disease, Banti's Disease, and Splenic Anæmia. — Hæmoglobin index 1 or less; no leucocytosis; leucocytes often in normal proportions—*i.e.*, there are no positive blood changes.

Pernicious Anæmia.—Hæmoglobin index greater than 1; no leucocytosis; white corpuscles in normal proportions or else lymphocytes relatively increased; in blood-films preponderance of megalocytes.

(*N.B.*—In all the above the red corpuscles may vary in size, show poikilocytosis, and present varying numbers of nucleated forms.)

Chlorosis.—Hæmoglobin index less than 1; white corpuscles in normal proportions. Not infrequently the anæmia consists entirely in diminution of the hæmoglobin, the red corpuscles being little, if any, below normal.

Typhoid Fever.—Leucopenia and relative increase in the small lymphocytes.

Malaria.—Leucopenia and relative increase in the large lymphocytes. Parasites present at certain times.

Abscess Formation and Lobar Pneumonia.—Leucocytosis and relative increase in the polymorphonuclear cells. The pus must be under pressure; there is no leucocytosis when the abscess is draining; nor in superficial suppuration, such as impetigo; nor in cases where the organisms are not confined, as in fungating endocarditis; nor must the suppuration be very viru-

lent—in cases of general suppurative peritonitis, for example, there is usually neither leucocytosis nor increase in the polymorphonuclear cells.

Cachexias without Suppuration. — Anæmia of the chlorotic type, with or without slight leucocytosis; relative increase of the small lymphocytes.

Morbus Cæruleus (congenital pulmonary stenosis). —The red cells are remarkably increased — up to 10,000,000 per cub. mm., for example. The hæmoglobin is also increased, but to a less extent. The leucocytes remain normal.

Mitral Stenosis, Chronic Bronchitis, and Emphysema, and other lesions associated with more or less cyanosis. The blood shows changes similar to those of morbus cæruleus, but to a less extent.

Altitude.—Those who live on mountains have simple polycythæmia, similar to that of mitral stenosis, but without cyanosis.

Tænia solium Tænia mediocanellata Hydatid disease Bothriocephalus latus	Chlorotic anæmia, with moderate eosinophilia.
Trichinosis Anchylostomiasis Bilharzia hæmatobia Filariasis Asthma, during the attacks Pemphigus Hydroa herpetiformis Herpes gestationis	Considerable eosinophilia, often up to 15 per cent., and sometimes 50 per cent. or more.

Filariasis: Parasites present at certain times.
Trypanosomiasis: Parasites present at certain times.

The Presence of Parasites—Malaria.—Films should be stained by Leishmann's method (p. 76),

though it is occasionally possible to see the parasites in the fresh blood under the microscope. The corpuscles should be searched as in making a differential leucocyte count, and hæmatozoa cannot be reported absent unless none have been found after half an hour. The best time for finding them is when the films are made at the beginning of an actual rigor. After quinine has been given none will be present.

The appearance of the parasites will vary with the stage of their development. Between the tertian and the quartan varieties there are but slight microscopic differences. Each begins as a small pale spherule situated excentrically within a red corpuscle. As it grows it expands at the expense of the protoplasm of the red cell, and produces dark pigment granules, which collect near its centre. This is the ring stage, at which it is commonly detected. In the stained film the remains of the red corpuscle is pink, and within it there is a bright blue parasite surrounded by an aggregation of almost black pigment granules (Fig. 49). The next stage, that of sporulation, is seldom seen, but not infrequently the parasite, instead of being entirely enclosed with the red cell, is elongated into a crescentic shape (Fig. 51), with pigment granules at its centre, and the ghost of a red corpuscle attached to its concave side. It appears that these crescents are not distinctive of any particular variety of malaria, but that either tertian or quartan parasites may occur in the crescentic form. A flagellate variety is also described, but it is only seen in fresh, undried blood that has been allowed to stand outside the body. It is possible that both the flagellate and the crescent

forms are really stages in the sexual cycle which the parasite passes through in the mosquito.

Filaria Sanguinis Hominis.—This is only found in persons who have been in the tropics. The mature worm occupies the pelvic lymphatics. Embryos find their way to the blood, and in the common form, *Filaria nocturna*, are present in the peripheral circulation at night only. In a rarer form, *Filaria diurna*, they occur in the peripheral blood only in the daytime. In rarer cases still, *Filaria perstans*, they are found both day and night. The embryo (Fig. 50), if present, is readily seen in films stained by Leishmann's method.

The Spirillum of Relapsing Fever (Fig. 52).—This occurs in the blood during the febrile attacks, and may be found in films stained by Leishmann's method. Each is from 10 μ to 40 μ long, but only 1 μ thick; in fresh blood it is highly motile.

Estimation of the Specific Gravity of the Blood.—Chloroform (specific gravity 1480), benzol (specific gravity 888), a sensitive urinometer graduated from 1020 to 1080, and a glass cylinder deep enough to take the urinometer, are required. Mix chloroform and benzol until the specific gravity of the mixture is 1058, which is that of normal blood. Prick the lobule of the ear; hold a small vessel containing some of the chloroform-benzol mixture under the ear, and allow a drop of blood to fall into it. Transfer this to the main bulk of mixed chloroform and benzol. Should the blood drop float, the specific gravity of the mixture is higher than that of the blood. Reduce it by adding more benzol. Should the blood drop sink, raise the

specific gravity of the mixture by adding chloroform. When the blood drop neither sinks nor floats, the specific gravity of the mixture is the same as that of the blood, and may be read off on the urinometer. A special specific gravity apparatus is required if very accurate readings are desired, because the urinometer graduations are for urines whose surface tension is different to that of a chloroform-benzol mixture.

Importance of Estimating the Specific Gravity of Blood.—The specific gravity rises when the blood becomes concentrated, and therefore affords a means of diagnosis between collapse and shock. Collapse follows great loss of fluid, the blood becomes concentrated, the specific gravity rises; treatment by transfusion is indicated. In shock there has been no loss of fluid, the specific gravity is not raised, transfusion is not indicated.

The specific gravity also affords a valuable check upon hæmoglobin estimations. Specific gravity and hæmoglobin rise and fall together. Approximately—

A sp. gr. of	1035	corresponds to	30	per cent. of	hæmoglobin			
,,	,,	1040	,,	,,	45	,,	,,	,,
,,	,,	1050	,,	,,	65	,,	,,	,,
,,	,,	1055	,,	,,	75	,,	,,	,,
,,	,,	1060	,,	,,	100	,,	,,	,,

Lipæmia.—In certain cases of diabetes mellitus the blood becomes pale and 'milky,' a condition which is in some cases due to fine globules of fat, in others to fine particles of a precipitated proteid circulating in the blood. A yellow colour of the retinal bloodvessels may suggest the onset of 'lipæmia.' Microscopic examination of a drop of blood may confirm it. The best method of staining fat droplets is to mix a fresh

drop of blood with a drop of the stain which is called Sudan III.* The fat particles become bright red, whilst nothing else present takes the stain. They indicate the imminence of coma, but in certain cases early recognition has led to the administration of large quantities of carbonate of soda in time to avert the fatal issue. Lipæmia has also been noted in severe alcoholism, in phosphorus-poisoning, and after fractures.

Widal's Reaction for Typhoid Fever.—The 'clumping reaction' is not so much a vital as a physical phenomenon. The test may be made with dead cultures, although, where possible, it is best to send the patient's blood-serum to a bacteriological laboratory for testing with living cultures.

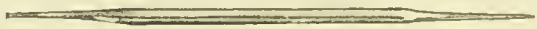

FIG. 53.—Widal Tube.

To obtain the serum, a glass pipette (Fig. 53), drawn into a capillary tube at either end, is needed. The patient's ear is pricked as in making a blood-film; into the little pool of blood which collects one end of the pipette is put, and blood immediately runs into it by capillary attraction. No suction is needed. If necessary, the rate of flow of blood into the pipette may be accelerated by keeping the free end below the level of the ear, but on no account should the blood be allowed to flow further than half-way along the tube. Now, holding the pipette horizontal, seal off the clear

* Alcohol (96 per cent.), 100 c.c.; Sudan III. (Grübler), to saturation; filter and add alcohol (50 per cent.), 75 c.c.; filter again.

capillary end in a spirit flame; as this end cools the blood will be drawn into the body of the pipette, making it now easy to seal off the other end in the same way. In about half an hour, when the blood has clotted, the tube may be turned vertically with its clear end downwards. The serum will run down into the clean end, comparatively free from red corpuscles, which otherwise would need to be got rid of by centrifugalizing. The tube is in most cases sent to a bacteriologist for the completion of the test, the usual process of which is briefly as follows:

The end of the pipette is broken off, and the serum received in a clean watch-glass. A platinum wire loopful is transferred to a cover-slip, and on to the same cover-slip are put nine separate loopfuls of normal saline. The drops are then mixed, so that the serum dilution is 1 in 10. A loopful of this diluted serum is transferred to another cover-slip, and to it nine loopfuls of salt solution are added. The second serum dilution is 1 in 100. A loopful of broth culture of typhoid bacilli is now put on to each of two clean cover-slips; to the first a loopful of the serum diluted 1 in 10 is added, making a dilution of 1 in 20; to the second, a loopful of the serum diluted 1 in 100, making a dilution of 1 in 200. Each cover-slip is inverted over a hanging-drop chamber, the edge having been vaselined to prevent evaporation, and the bacilli are looked at in the hanging drop under high power. If the test be positive, the bacilli will adhere to one another in batches or 'clumps,' instead of being evenly distributed. The test gives positive proof of typhoid fever if the clumping occur within half an hour with a serum dilu-

tion of 1 in 200. If the clumping take place with a dilution of 1 in 20, and not with 1 in 200, typhoid fever may, or may not, be present. The reaction is seldom fully positive until after the end of the first week of the fever.

Examination for Bacteria in Blood.—Circulating blood seldom contains enough bacteria to be detected in films stained bacteriologically by the methods described on pp. 101-113. In most cases it is necessary to obtain enough blood to send to special laboratories for cultivation. The best way to obtain it is direct from a vein in the arm by means of a syringe. The skin must be made surgically clean, but all antiseptic must be removed with boiled water, or else the organisms will be prevented from growing. The arm is prepared as for venesection, but, instead of incising the skin, the needle of the syringe is plunged obliquely into the distended vein. The syringe and needle must be sterile, but free from antiseptic, and to prevent the blood from clotting a few c.c. of 1 per cent. solution of citrate of potash should be present in it to start with. Another method is to collect blood in a series of sterilized Widal tubes from the lobule of the ear (p. 92), after rendering the latter aseptic. This is far less drastic than the former, but it does not yield so much blood, and contamination is more likely to occur. The more blood obtained, the more likely are organisms in it to grow ; about 10 c.c. is a usual amount to take. The kind of cases in which the procedure is required are those of septic infection or suspected fungating endocarditis, when identification of the organism will lead to the use of the proper vaccine or serum in

treatment. In typhoid fever it is said that the *Bacillus typhosus* may be found in the blood early in the illness, thus affording a possible means of early diagnosis.

The Opsonic Index of the Blood.—Opsonins are the substances in the blood-serum which control the phagocytic power of the leucocytes. There appear to be different opsonins for different bacteria, so that each person has one opsonic index to tubercle bacilli, another to staphylococci, and so on. To determine the opsonic index against any particular organism it is necessary to obtain blood-serum from the patient by the same process as for Widal's test (p. 92), strict asepsis being observed. The specimen is then sent to a bacteriological laboratory, where they prepare—

1. Serum from a normal person.

2. Fresh leucocytes, obtained as follows: Drops of blood from a finger are received into normal saline containing a little citrate of soda. The mixture is centrifugalized, and the supernatant fluid poured off. The mixed red and white corpuscles at the bottom are then shaken up with fresh normal saline, and re-centrifugalized. The supernatant fluid is poured off; the deposit furnishes the leucocytes required.

3. An emulsion of the particular organism against which the opsonic index is to be taken.

The procedure is briefly as follows: Upon each of two capillary glass tubes, fitted with a rubber teat at one end, a mark is made about an inch from the other end. The rubber teat is compressed, and the end of one of the pipettes is put into the washed corpuscles; by slowly relaxing the pressure on the teat, the latter are drawn up until they reach the mark on the tube.

A bubble of air is then drawn in, and the end of the pipette dipped into the patient's serum. The latter is drawn up till it reaches the mark, another bubble of air is drawn in, and then emulsion of bacteria is drawn up to the mark in the same way. The tube now contains equal quantities of—(*a*) washed corpuscles; (*b*) patient's serum; (*c*) emulsion. The contents are mixed by expelling them on to a slide, reaspirated high up into the capillary tube, and the latter then sealed above and below. With the other pipette a precisely similar operation is carried out, substituting normal serum for that of the patient. Both sealed tubes are now put into the incubator for fifteen minutes. Blood films are then made from the contents of each tube, and appropriately stained. The leucocytes will be found to have taken into themselves a certain number of bacteria. The numbers so taken in by 50 polymorphonuclear cells in each slide are counted. Suppose in the preparation in which the patient's serum was used 50 leucocytes had taken in 85 bacteria, and that on the other 50 leucocytes had taken in 170, the patient's opsonic index for the organism in question would be $\frac{85}{170}$, or 0.5.

Estimation of Calcium Salts in Blood.—Calcium salts have a very important rôle in the coagulability of blood. The possible importance of calcium in hæmophilia, menorrhagia, scurvy, blood diseases, and so forth, is therefore great. The following clinical method of estimating calcium in the blood, and of gauging the effects of administering calcium by the mouth, has been recently described:

Bell's Method.—The small capsule (Fig. 54 [1]) contains 200 cub. mm. of 1 in 30 aqueous solution of oxalic acid; when about to be used it is broken at A. The patient's ear is pricked, 100 cub. mm. of blood are drawn into the pipette (Fig. 54 [2]), immediately expelled into the oxalic acid, and the capsule sealed. Its contents become laked; ten minutes later they are ready for further dilution, though the next steps may be postponed for hours if necessary. The bulb is broken at B, and 250 cub. mm. of the following reagent added to it:

Acetic acid (1 per cent.)..	95 parts
Glycerine	5 ,,

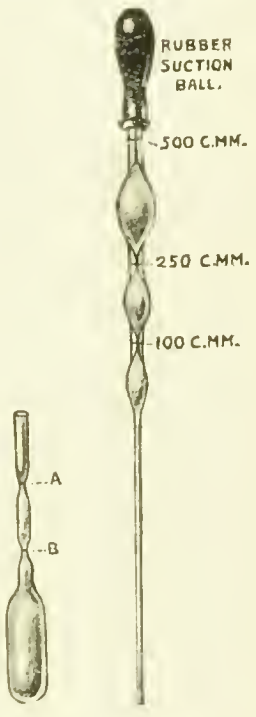

Fig. 54.—Bell's Apparatus for Estimating Calcium in Blood.

After thorough shaking, 100 cub. mm. are transferred to a vessel containing 500 cub. mm. of *distilled* water. This makes the final mixture, a drop of which is transferred to a Thoma-Zeiss hæmocytometer (p. 59), and the number of calcium oxalate crystals counted precisely as in making a blood examination. A large number of squares should be counted. One crystal per square is equivalent to 1 in 6,000 CaO. Healthy adults have between 1 in 4,000 and 1 in 5,000 CaO in their blood.

To Estimate the Coagulability of the Blood.—This requires a special apparatus, termed a coagulometer. Full directions are issued with the instrument.

CHAPTER III

EXAMINATION OF THE SPUTUM

I. NAKED-EYE CHARACTERS.

CERTAIN macroscopic appearances of the sputum are typical.

In bronchitis it is at first small in amount, frothy, and contains small, clear, sticky pellets of mucus. Later the expectoration, though still frothy upon the surface, has a thick, opaque, yellowish deposit of muco-pus.

In phthisis with cavitation numular sputum is distinctive—heavy airless lumps of muco-pus, each of which, if expectorated on to a dry surface, flattens out into a circular meniscus, which does not coalesce with its neighbours; hence called 'numular,' or coin-like. If expectorated into carbolic lotion or other fluid, each more or less spherical, airless mass sinks to the bottom, but remains distinct from its neighbours. Streaks of red blood occur in the earlier stages; later, blood-clots resembling in shape the lung cavities. It must not be forgotten that streaks and clots of blood result also from adenoids, mitral stenosis, fibroid lung, whooping-cough, and severe bronchitis.

In fibroid lung with Bronchiectasis the amount of sputum may be great, up to as much as 1 pint or more in the twenty-four hours. It often smells very

foul. Usually expectoration occurs at comparatively long intervals, when a large quantity is coughed up at a time. Received into a specimen-glass, it settles into layers; upon the surface, frothy serum; at the bottom, a thick sediment of muco-pus; between, clear serous fluid.

In cases where an empyema has ruptured into a bronchus the sputum is very similar to that of bronchiectasis.

In lobar pneumonia the sputum is termed 'rusty.' This name applies to its usual colour, a dull red-brown. The colour is not constant, for it is due to blood, and may be any hue that a bruise may be, from bright red to dull red, brown, bluish, green, or yellow. The chief characteristic of pneumonic sputum is its viscosity. So glairy is it that, if expectorated into a dry porringer, the latter may be turned upside-down, and yet the sputum will only very slowly slide along its surface.

In plastic Bronchitis, a rare condition, casts of the bronchioles occur. Similar casts may be found in cases of diphtheria in which membranous exudate has extended into the tubes.

Anchovy-sauce-coloured expectoration is rare, but is almost pathognomonic of hepatic abscess ruptured into the lung.

No other types of sputum can be called characteristic. In infarction of the lung it is said to resemble 'prune-juice,' in growth of the lung 'red-currant jelly,' but neither is constant. Morning expectoration of thick phlegm stained black by smoke and dust occurs in healthy persons who live in towns or smoky atmospheres.

II. MICROSCOPICAL CHARACTERS.

The Sputum in Phthisis.

It is in cases of phthisis, suspected or certain, that investigations of the sputa are so important. The following points should be attended to:

1. The amount. Large quantities indicate cavitation or bronchiectasis.

2. The character. Numuli indicate cavitation.

3. The presence of blood. Streaks in early phthisis; clots when there is cavitation.

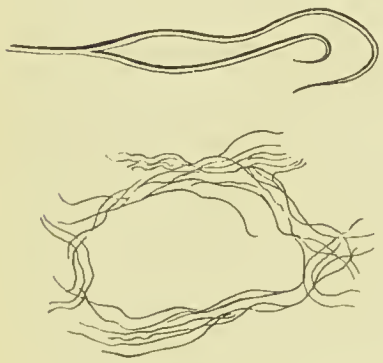

FIG. 55.—Elastic Fibres from Lung, the lower less magnified, and showing Alveolar Arrangement.

4. The presence of elastic fibres.

5. The presence of tubercle bacilli, and whether few, many, or very many.

6. The presence of other bacteria, particularly staphylococci and streptococci.

Elastic Fibres.—To the sputum add excess of 10 per cent. caustic soda, and boil for five minutes. All the organized elements except the elastic fibres and adventitious hairs are destroyed. Pour the swollen gelatinous residue into 3 inches of water in a specimen-glass, stir well, and allow to settle. If necessary, centrifugalize the deposit. Transfer some of the deposit to a slide, cover, and examine under a low power. Elastic fibres (Fig. 55) are sharply defined,

double-contoured threads, sometimes single, sometimes branched, and occasionally arranged in a meshwork corresponding in outline to a pulmonary air vesicle.

Importance.—They indicate a serious condition of active lung disintegration, such as occurs in advancing phthisis, gangrene, or pulmonary abscess.

Tubercle Bacilli.—Make a film. It is essential that the sample taken for this purpose should be from the interior of a numulus. It is best picked out by a fine pair of forceps, and it should be smeared *fairly thickly* over a clean slide and allowed to dry in the air. Disinfect the forceps with heat. In many cases it is sufficient to make a film in this way without previous treatment of the sputum, but where tubercle bacilli are few, they may not be found without first breaking up the numuli as follows:

Into a small beaker put 10 c.c. of the sputum and 20 c.c. of water; add 5 drops of 10 per cent. caustic soda, boil gently for five minutes, stirring well; add 100 c.c. of cold water, stir well, and allow to settle. Centrifugalize the deposit, and proceed to make films. After such treatment bacilli will adhere better if the slides be albuminized (see p. 20).

Having made and dried the film, fix it by passing it slowly through a Bunsen flame five times, film upwards, then stain by the **Ziehl-Neelsen method**, for which the following reagents are required:

1. *Carbol Fuchsine Stain.*

Saturated alcoholic solution of fuchsine	10 c.c.
Carbolic acid crystals	5 grammes
Distilled water to	100 c.c.
Add absolute alcohol	10 ,,

2. *Sulphuric Acid.*

25 per cent. solution.

3. *Carbol Methylene Blue Stain.*

Methylene blue	..	..	1·5 grammes
Carbolic acid crystals	..	..	5·0 ,,
Distilled water to	..	..	100·0 c.c.
Add absolute alcohol	..	..	10·0 ,,

Warm some carbol fuchsine in an evaporating basin until it begins to steam freely, and immerse the film in it for five minutes; then wash in tap-water, and immerse in 25 per cent. sulphuric acid; the latter changes the red colour to orange yellow, and much of the stain is discharged. In one minute wash off the acid with tap-water. The red colour will reappear. If it be a faint pink, enough stain has been removed; if a darker red, return the film to the sulphuric acid until, on washing with tap-water, only a faint pink remains. The tendency is to remove too little stain rather than too much. Now blot with filter-paper and transfer to carbol methylene blue for three minutes; wash in tap-water; blot; allow to dry in the air; examine under $\frac{1}{12}$-inch oil-immersion lens with a good Abbé condenser.

The principle of the above method is as follows: Tubercle bacilli take up stain with difficulty, but once they are stained it is difficult to unstain them. Cold carbol fuchsine will not stain them, warm will; but at the same time all the other structures are greatly overstained. The sulphuric acid corrects this by removing the fuchsine from everything except the tubercle bacilli. Leprosy bacilli and smegma bacilli are the only other organisms which retain the stain in

the same way. Carbol methylene blue counterstains the remaining structures, so that tubercle bacilli stand out red in a blue field.

Each measures about 3 μ in length, or less than half the diameter of a red corpuscle, and they are exceedingly thin. They frequently have a beaded appearance, though they are said not to form spores. They may occur singly or in pairs joined end to end (see Plate II., Fig. 59), or in clusters of half a dozen or more.

Importance.—Tubercle bacilli are never adventitious; their presence means tuberculous trouble. It is not possible, however, to say that tuberculosis is absent because no organisms have been found. Careful and repeated search may be needed before such negative evidence can be relied on. In acute miliary tuberculosis of the lung they may not be found at all. On the other hand, presence of large numbers is not a hopeless sign.

Other Pathogenic Bacteria in Phthisical Sputum—Staphylococci.—Each is about 1 μ in diameter, arranged in clusters and irregular clumps (Plate II., Fig. 60).

Streptococci.—Each is about 1 μ in diameter, arranged in short or long strings (Plate II., Fig. 61).

Diplococci.—Each is about 1 μ in diameter, arranged in pairs, with an unstained capsule round each pair (Plate II., Fig. 63).

Importance.—As a general rule, when many tubercle bacilli are present, there are few other organisms, and *vice versa.* Many of the severe symptoms of phthisis are due to staphylococci and streptococci. When such

secondary infection has occurred, cachexia and toxic symptoms increase, pyrexia is more constant, and the prognosis, as a rule, worse. In this respect, the presence of streptococci is more serious than that of staphylococci only. Diplococci have no particular significance in such cases ; they may, indeed, be found in perfectly healthy persons.

Non-Pathogenic Bacteria in Phthisical Sputum.—**Bacteria** of various kinds occur ; their names are not important.

Tetrads or **Sarcinæ**, consisting of cocci arranged in fours, or multiples of four (Fig. 56). These indicate putrefaction in the cavities in the lung, and are therefore a bad sign, though in themselves harmless. They occur in bronchiectasis as well as in phthisis.

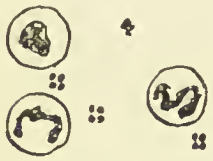

FIG. 56.—Tetrads and Pus Cells.

Pus Cells and Mucus in Phthisical Sputum.—The former are chiefly polymorphonuclear leucocytes, the latter structureless streaks and wisps. Both are stained blue.

Importance.—In the earlier stages of phthisis mucus predominates ; later pus cells may be extremely numerous, and indicate suppurating cavities.

In addition to the above constituents of a film, large squamous cells may occur ; these are derived from the buccal mucosa, and have no pathological significance.

The Sputum in Lobar Pneumonia.

The viscid character of the sputum is almost pathognomonic ; microscopical examination is confirmatory,

but not essential. Lobar pneumonia is caused by more organisms than one. Films stained either with carbol methylene blue (p. 108), carbol fuchsine (p. 108), or by Gram's method (p. 108), will show many pus cells, some red corpuscles, streaks of mucus, probably groups of staphylococci and strings of streptococci, and numbers of one or other of the following bacteria:

Diplococci Pneumoniæ of Fraenkel (Plate II., Fig. 63).—This is the causative agent in most cases. Each organism is a pointed coccus about 1 μ in longest diameter; they occur in pairs, within a capsule which remains unstained by ordinary methods. They stain with carbol fuchsine and with carbol methylene blue, and retain the stain by Gram's method. Their capsule may be stained by the following process:

Capsule Stain.

Dahlia	0·5 gramme
Methyl green (oo crystals)	1·5 grammes
Saturated alcoholic solution of fuchsine	10 c.c.
Distilled water to	200 ,,

Immerse a film in the stain for five minutes; wash gently in distilled water, and dry.

Pneumo-bacilli of Friedländer.—These are short rods occurring in pairs within a capsule. They stain with carbol fuchsine and with carbol methylene blue, but not with Gram's method. They are rare, and difficult to identify by the microscope alone.

Influenza Bacilli.—These are exceedingly minute rods, 1·5 μ long by 0·3 μ broad. They are not arranged in pairs, and have no capsule. They stain with carbol fuchsine and with carbol methylene blue, but not by Gram's method.

Importance.—Each of these organisms may occur in the sputum of patients who are not suffering from pneumonia; more stress is to be laid upon the viscidity of the sputum than upon the micro-organisms it contains, unless possibly in differentiating influenzal pneumonia from the ordinary form.

Charcot-Leyden Crystals. — These consist of phosphate of spermin, and are visible in fresh sputum under the high power as colourless crystals of elongated diamond shape (Fig. 57). They are not pathognomonic of any particular affection, nor are they common. They occur in some cases of asthma, but not in all. They have been found in bronchiectasis and in chronic phthisis. Since they have also been obtained from seminal fluid, and from leucocythæmic blood, they are regarded as products of cell disintegration.

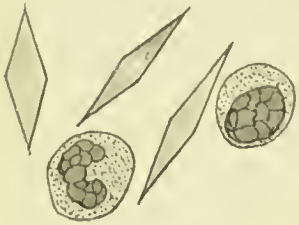

Fig. 57.—Charcot-Leyden Crystals and Pus Cells.

Curschmann's Spirals. — These may be seen in fresh sputum under the low power, and may even be visible to the naked eye. Each consists of a central core, round which is woven a thick spiral meshwork of delicate fibres (Fig. 58). Apparently they are mucoid casts of the finest bronchioles. They have been found in broncho-pneumonia, in bronchiectasis, in capillary

Fig. 58.—Curschmann's Spiral.

bronchitis, in asthma. If any significance can be attached to them at all, other than that they indicate affection of the bronchioles, it is in asthma, where their presence may prove that the patient has bronchial asthma as distinct from other forms, such as uræmic or cardiac.

CHAPTER IV

EXAMINATION OF PUS

The chief point to be determined is the variety of organism present. Smear out some of the pus in a thin layer upon a clean slide; allow the film to dry spontaneously; when quite dry, fix it by passing it five times through a Bunsen or spirit flame. Stain by one or other of the following methods:

1. **With Carbol Methylene Blue** (p. 102).—Immerse the film in the stain for five minutes; wash gently in water for a few seconds; blot, and dry in the air.

2. **With Carbol Fuchsine** (p. 101).—Immerse the film in the stain for three minutes; wash vigorously in water for thirty seconds; blot, and dry in the air.

This and the preceding method will stain all the organisms described below except the tubercle bacillus. Carbol fuchsine readily overstains; the reverse is true of carbol methylene blue; in each case the carbolic acid acts as a mordant, and is essential.

3. **By Gram's Method.**—The following solutions are required:

(*a*) *Aniline Water:*

Aniline oil	5 c.c.
Water	100 ,,

Shake up together in a bottle; the water becomes impregnated with the aniline oil, but globules of the latter remain undissolved. The water must therefore be filtered when used.

(*b*) *Gentian Violet Stain:*

Saturated solution of gentian violet in absolute alcohol.

(*c*) *Gram's Iodine Solution:*

Iodine	1 gramme
Potassium iodide	2 grammes
Distilled water	300 c.c.

Fill a suitable small vessel three-parts full of filtered aniline water; add 5 to 10 drops of gentian violet solution; immerse the film in the mixture for five minutes. Wash off the free stain with water; transfer the film to Gram's iodine solution; previously violet, it here becomes a deep slate colour. After one minute remove it from the iodine, and wash with alcohol, which removes much of the colour and makes the film pale grey. Wash in water again; the film should now become a quite pale violet colour. If the tint be at all deep, insufficient stain has been removed, in which case repeat the iodine process; then blot off the water with filter-paper, and allow the film to dry in the air.

Some organisms are Gram-positive—that is to say, they retain the gentian violet in spite of treatment with iodine; others are Gram-negative. The method is therefore of considerable assistance in diagnosis.

Gram-Positive Organisms.	*Gram-Negative Organisms.*
Staphylococci.	Gonococci.
Streptococci.	Pneumobacilli.
Pneumococci.	Bacillus coli communis.
Anthrax bacilli.	Bacillus typhosus.
Diphtheria bacilli.	Bacillus mallei.
Tetanus bacilli.	Cholera vibrios.
Actinomycosis (parts).	Plague bacilli.
	Diplococcus intracellularis.

4. **By the Ziehl-Neelsen Method** for tubercle bacilli (see p. 101).

5. **By the Capsule Stain** for pneumococci and for pneumobacilli (see p. 105).

Examine the stained film with $\frac{1}{12}$-inch oil-immersion lens, using an Abbé condenser; without the latter, bacteria are difficult to see. There is no need to use Canada balsam and a cover-glass unless the film is to be kept. Occasionally, and particularly in tuberculous pus, no organisms may be present. More often bacteria will be found. Identification can seldom be made with absolute certainty without the cultural methods of a special laboratory. The following, however, are the microscopical characters of some organisms that may be met with :*

Staphylococcus Pyogenes.—Each is spherical, about 1 μ in diameter; it occurs in irregular clusters or masses. Three varieties are described: (*a*) **Albus**; (*b*) **aureus**; (*c*) **citreus**, according to the colour of the cultures on gelatine. Under the microscope all look alike (Plate II., Fig. 60). It is of frequent occurrence in subcutaneous abscesses, sputum from

* It often happens that pus contains non-pathogenic as well as pathogenic bacteria; much experience is needed in distinguishing these. For descriptions of them a larger book upon the subject should be consulted.

lung cavities, acne, boils, and, indeed, most forms of suppuration. It is usually less virulent than other pyogenic organisms.

Streptococcus Pyogenes.—Each is spherical, about 1 μ in diameter; it forms chains, which may be long or short, the organism being called in the one case **Streptococcus longus**, in the other **Streptococcus brevis** (Plate II., Fig. 61).

It is a virulent organism in many cases, not so often found as staphylococcus, but a more serious infection. It may occur in simple abscesses, phthisical sputum, pyorrhœa alveolaris, empyemata, suppurative peritonitis, erysipelas, fungating endocarditis, osteomyelitis, septic conditions of the uterus, pyæmia, septicæmia.

Gonococcus.—Each is rather larger than a staphylococcus, and is kidney-shaped rather than spherical; they occur in pairs, but have no capsule, and they are found *inside* the pus corpuscles as well as outside (Plate II., Fig. 62).

Its presence in urethral pus may elucidate a doubtful case of urethritis, though it dies out in chronic cases. It may also occur in gonorrhœal ophthalmia, synovitis, abscesses, and infective endocarditis. It is often of great medico-legal importance, in connexion with stains on clothes, vaginal discharges in children or young girls, and so forth.

Pneumococcus of Fraënkel.—Each is 1 μ in diameter, not quite spherical, owing to a sharp, lancet-shaped projection. They are arranged in pairs, with the points in opposite directions, and each pair is surrounded by a capsule (Plate II., Fig. 63).

It is the chief cause of lobar pneumonia, in the

sputum of which it may be found in abundance. It is, however, sometimes found in healthy persons or in phthisical sputum where there is no pneumonia. It occurs in many cases of empyemata, and more rarely in pneumococcal peritonitis, arthritis, meningitis, or infective endocarditis.

Pneumobacillus of Friedländer (see p. 105).

Diplococcus Intracellularis.—Each is 1 μ in diameter. They are arranged in pairs, with no capsule, and occur both inside and outside the pus cells. They do not retain the stain by Gram's method, in this respect differing from pneumococci, but resembling gonococci ; they differ from the latter in not being kidney-shaped ; they give rise to posterior basal and to epidemic cerebro-spinal meningitis, and are present in the pus beneath the pia mater. They may sometimes be found in the cerebro-spinal fluid obtained by lumbar puncture.

Bacillus Coli Communis.—Each is a short rod, with blunt, rounded ends, measuring 3 μ by 0·5 μ. In the fresh state it is motile by means of flagella, which require special and somewhat difficult methods of staining. It does not form spores. Occurring abundantly in the large intestine, it is there harmless. Escaping to other tissues, it may become very virulent, and is found in many cases of appendicular abscess, general suppurative peritonitis, vulvitis, and cystitis.

Bacillus Typhosus, or Eberth's Bacillus.—This is indistinguishable from the *Bacillus coli communis* except by very special methods of culture. Microscopically the two are identical.

Diphtheria Bacilli, or Klebs-Lœffler Bacilli.—These are slender rods about 3 μ by 0·4 μ, but varying considerably. They are non-motile, and do not form spores, though they often show beads of lighter and darker staining which resemble spores (Plate II., Fig. 64). They often show a tendency to form short chains, and one end is frequently swollen more than the other, so that a club-like appearance is presented. It is very important in a doubtful case of sore throat to examine for the bacilli. Depress the patient's tongue with a spatula whilst he breathes through his mouth; collect a little of the suspected matter from the tonsil upon a pledget of cotton-wool; make a film from this; fix it by heat, and stain either by Gram's method or, better still, by Neisser's stain.

Neisser's Stain for Diphtheria Bacilli.

Methylene blue (Grübler's)	1 gramme
Rectified spirit	20 c.c.
Distilled water	950 ,,
Glacial acetic acid	50 ,,

Contrast Stain.

Vesuvin	2 grammes
Distilled water	1,000 c.c.

Boil; allow to stand; filter; and store for use.

Immerse the film in Neisser's stain for four seconds; rinse rapidly in distilled water; immerse in the contrast stain for four seconds; rinse in distilled water; blot and dry. Diphtheria bacilli will be stained brown, with an occasional bright blue granule in their interior. Their presence may thus be established in a few minutes, and under conditions when there may be no facilities for employing cultural

methods. It is, nevertheless, always advisable to have a cultivation made at the same time, in order that the diagnosis may be confirmed or otherwise by examination of the culture after twenty-four hours' incubation.

Many cases of diphtheria need no such examination to assist the diagnosis. Many others cannot be diagnosed from septic sore throat or follicular tonsillitis without it. On the other hand, diphtheria bacilli sometimes occur in the throats of people who are seemingly healthy, so that, without clinical signs as well, the mere presence of the organisms does not constitute diphtheria.

Anthrax Bacillus.—Each is a characteristic and comparatively large organism, 7 μ long and 1·2 μ broad, non-motile, spore-forming, and many may be joined end to end in a thread-like chain (Plate II., Fig. 65).

Anthrax infections occur mainly in those who work with raw foreign hides or wool. The common lesion is a local 'malignant pustule' on the face or hand. Early detection of the organisms is all-important. Whilst they are local to the pustule, serum treatment, with or without excision, saves the patient. After infection of the blood-stream the disease is beyond control.

Tetanus Bacillus.—Each is a slender rod, 4 μ long by 0·4 μ broad. They do not form chains as a rule, and, when fresh, are very slightly motile. Their chief characteristic is the formation of a large bulging spore at one end, which gives them a 'drum-stick' shape (Plate II., Fig. 66). It is by the clinical signs

that the disease is diagnosed. The discovery of the organism has its chief value in determining where the inoculation has been, and, consequently, the part requiring surgical attention. The bacillus is anaërobic, and occurs locally in some septic wound. Along with it numbers of other organisms are usually present.

Actinomycosis.—This is rare in man. It affects least rarely the lower jaw, and very rarely the cæcum, the liver, or the lung. To the naked eye the pus may contain minute granules, not bigger than a small pin's head, usually semi-translucent and of a greenish-grey. Quite rarely their colour is an orange yellow. Under the high power each granule is seen to have three parts, namely: (*a*) A central *mycelium* of fine interlacing threads; (*b*) *cocci*, or small spore-like bodies, intermingled with the mycelium; (*c*) *clubs*, the swollen, pear-shaped ends of filaments which project radially outwards from the surface, and give the 'rayed' form to the mass (Plate II., Fig. 67).

The mycelium and cocci stain both with the ordinary aniline dyes and by Gram's method; the clubs stain with the former, but not by Gram's process.

Notwithstanding the rarity of the affection, its diagnosis from other forms of suppuration is all-important, because potassium iodide may cure it without need for operative measures.

Glanders.—The *Bacillus mallei* is a minute rod, 3 μ by 0·4 μ, with a tendency to form short chains. It is non-motile, does not form spores, and does not stain by Gram's method.

It occurs sometimes in those who have to do with horses. The lesions are in the skin or in the lung, and resemble those of tubercle. Discovery of a bacillus in the caseous pus should be followed by cultural identification of the organism.

Cholera Vibrio. — This is found in the rice-water stools. It is a bacillus curved like a comma (Plate II., Fig. 68)—broader at one end than the other, with a flagellum at the narrow end, by means of which it is motile when alive. It is very small, 1·5 μ by 0·5 μ, and there is a tendency for two to join end to end, with their curves opposite ways, like the letter S.

Plague Bacilli.—These are small oval rods with rounded ends which stain deeply, whilst the central part remains unstained, a characteristic to which the term 'pole-staining' has been given (Plate II., Fig. 69). They do not form spores, are non-motile, and occur singly as a rule, though they may form chains. They may be looked for in the affected glands or buboes, or, in pulmonary cases, in the sputum.

For **Tubercle Bacilli**, see p. 101.

For the **Amœba of Dysentery**, see p. 136. This gives rise to hepatic abscess, the pus from which has a characteristic chocolate colour. When obtained from the interior of the abscess, the pus is often sterile; but if scrapings from the abscess wall be taken, the amœba is usually demonstrable. Dysentery itself has more than one causal factor; the variety due to Shiga's bacillus does not tend to be followed by hepatic abscess.

The Spirochæta Pallida.—Evidence is accumu-

lating to show that this is the causal organism in syphilis. There are many known spirochætæ, but the *S. pallida* is distinct from the others. It is a motile spirillum, about 7 μ long, and extremely thin, being less than 0·25 μ across: hence the difficulty in seeing it. It has from six to fourteen abrupt, deep, regular, narrow corkscrew twists. It stains with Leishmann's stain (p. 76), or, better, with a modification of this, sold as **Giemsa's stain.** This is used as follows: The film is fixed in alcohol, and then covered with Giemsa's stain, to 15 c.c., of which 10 c.c. of distilled water have been added; it is left for at least an hour, and may be left all night, then washed in distilled water and dried. *S. pallida* will now be rose-pink, whereas other spirochætæ would be bluish or purple. It has been found most often in chancres and in secondary lesions, such as condylomata and mucous ulcers; it is less common in tertiary lesions, but has been detected in them and in congenital cases. The surface of the lesion should be cleaned and scraped with a sharp spoon, films being made from the blood-stained scrapings obtained from the deeper parts.

Vincent's Angina.—This is a condition which resembles diphtheria in the appearance of the lesions and the epidemic character of the complaint, but differs from it in not having its serious sequelæ, and in not requiring the serum treatment. When examining for diphtheria bacilli in these cases, Vincent found two characteristic organisms (Plate II., Fig. 70)—(*a*) a spirillum, and (*b*) a fusiform bacillus. The former by itself is not unlike some non-pathogenic spirilla,

but the fusiform bacillus is readily recognized; it is a slender, slightly curved bacillus, tapering off to a point at each end. It is stained slowly by carbol methylene blue, so that the film should be immersed for fifteen minutes instead of five. It is also stained by Leishmann's method.

CHAPTER V

EXAMINATION OF THE GASTRIC CONTENTS

A. CHEMICAL EXAMINATION.

THE **apparatus** required is—

One glass funnel, 6 inches in diameter; one glass funnel, 2 inches in diameter; two glass beakers of 300 c.c. capacity; one glass flask of 1,000 c.c. capacity; one burette for decinormal soda solution; one pipette, 100 c.c.; one pipette, 10 c.c.; four small evaporating basins of 50 c.c. capacity; one small flat porcelain slab; test-tubes; fine muslin for straining; filter-papers for the smaller funnel; litmus-papers, blue and red; a Bunsen burner, or spirit lamp; an iron tripod; reagent bottles, conveniently 2 or 4 ounce capacity; one big bottle for standard soda solution; and one flat saucepan, about 6 inches in diameter and about 2 inches deep, for use as a water-bath.

The following **reagents** are necessary:

Gunsberg's Reagent.—A test for free hydrochloric acid:

Phloroglucin	2 grammes
Vanillin	1 gramme
Rectified spirit	100 c.c.

Keep the bottle stoppered and not exposed to bright

light. The solution need not be freshly prepared, but it loses its delicacy as a test in time, so that fresh should occasionally be made.

Congo-red Test Papers.—A test for free acid, organic and inorganic:

Congo red	0·1 gramme
Water	100·0 c.c.

Into the solution dip filter-paper, allow the latter to dry, cut it into strips, and store ready for use.

Uffelmann's Reagent.—A test for lactic acid:

Carbolic acid	3 grammes
Distilled water	100 c.c.

To a little of this solution, about 1 inch in a test-tube, add 1 or 2 drops of liquor ferri perchlor. (B.P.); an amethyst blue colour results. This is the complete reagent, but it does not keep, so that the ferric chloride and carbolic acid solutions should be kept in separate bottles, and mixed when required.

Decinormal Soda Solutions for the Estimation of Acidity.—A decinormal solution is such as contains one-tenth part of the molecular weight, in grammes, of a univalent substance, dissolved up to 1,000 c.c., usually in water. The cheapest method in the case of soda is to use sticks of caustic soda, but they are difficult to weigh accurately, because they absorb water from the air. The molecular weight of NaHO is $23+1+16=40$, so that 4 grammes of solid caustic soda, dissolved up to 1,000 c.c. in water, makes a decinormal solution.

The best plan is to make the solution as accurately as possible; then to titrate it with a known solution of

DESCRIPTION OF PLATE II.

FIG. 59.—Tubercle Bacilli and Pus Cells.
,, 60.—Staphylococci.
,, 61.—Streptococci.
,, 62.—Gonococci.
,, 63.—Pneumococci.
,, 64.—Diphtheria Bacilli.
,, 65.—Anthrax Bacilli.
,, 66.—Tetanus Bacilli.
,, 67.—Actinomyces.
,, 68.—Cholera Vibrio.
,, 69.—Plague Bacilli.
,, 70.—Vincent's Angina Organisms.

Plate II.

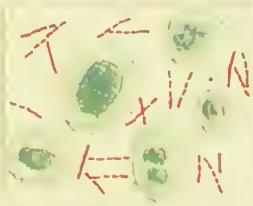

Fig. 59.

Fig. 60.

Fig. 61.

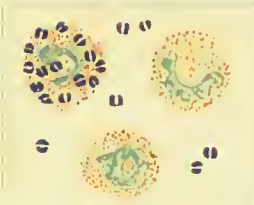

Fig. 62.

Fig. 63.

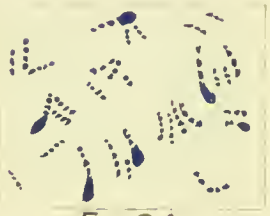

Fig. 64.

Fig. 65.

Fig. 66.

Fig. 67.

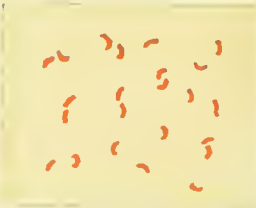

Fig. 68.

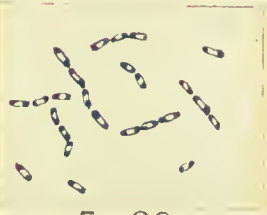

Fig. 69.

Fig. 70.

sulphuric acid, and thus determine its actual strength. Provided the strength of the soda solution be accurately known, it matters little whether it be absolutely decinormal or not.

The following is an accurate but more expensive method: Weigh a small test-tube, dried and corked; with a clean knife cut the outside crust from one or more pieces of pure sodium; transfer the bright metal to the test-tube; cork, and weigh again. Deduct the weight of the test-tube and cork to find that of the sodium, transfer the latter to a clean flask containing about 200 c.c. of absolute alcohol. It dissolves with evolution of heat, but with less violence than in water; it forms sodium ethylate, which can then be diluted with distilled water to the calculated volume and kept for use. Every 2·3 grammes of sodium must be diluted up to 1,000 c.c. to make a decinormal solution, of which 1 c.c. corresponds to 0·0023 gramme of sodium, or to 0·004 gramme of NaHO, or to 0·00365 gramme of HCl.

Phenolphthaleïn Solution.—The indicator in estimating acidity:

Phenolphthaleïn	0·2 gramme
Rectified spirit	60·0 c.c.
Distilled water to	100·0 ,,

The solution is colourless in the presence of even weak acids, and is turned rose red by alkali.

Discs of Hard-boiled White of Egg for Testing for Pepsin.—Boil an egg for twenty minutes; let it get cold; remove the shell; peel the white from the yolk, cut the former into strips, and punch out discs

with a cork-borer. Put the discs into glycerine in a small bottle ready for use.

The following reagents are not necessary, but may be employed as additional or alternative tests for free hydrochloric acid :

Boas's Reagent.

Resorcin	5 grammes
White sugar	3 ,,
Rectified spirit	100 c.c.

Tropæolin OO.

Methyl orange	0·2 gramme
Rectified spirit	25·0 c.c.
Distilled water to	100·0 ,,

Methyl Violet, or Pyoktanin.

Methyl violet	0·05 gramme
Distilled water	100·0 c.c.

The Method of Procedure.—First, *strain the vomit* through fine muslin, using the large funnel and flask. Do not use filter-papers, as these alter the acidity of the filtrate by allowing certain of the acid substances to pass through with greater readiness than others.

Next, *test the filtrate with blue litmus-paper.* The vomit may be alkaline. On the other hand, if blue litmus be turned red, the 'acidity' may be due to one or more of four causes :

(*a*) Acid salts (acid sodium phosphate).

(*b*) 'Combined' hydrochloric acid : that is, HCl molecularly combined with the proteid. In normal digestion after a proteid meal, the first part of the HCl secreted combines loosely with the proteid molecules ; during this time salivary digestion of carbohydrates continues, lactic acid is usually found, and it

is not until about an hour later that enough HCl has been secreted to leave a surplus 'free.' From this time onwards more 'free' HCl is present and less lactic, until the HCl reaches 0·2 per cent. and lactic acid is absent.

(*c*) Organic acids, chiefly lactic, with traces of acetic and butyric.

(*d*) 'Free' hydrochloric acid.

To Determine the Causal Factors of the Acidity.

Test with a Congo-red Paper.—If the colour be not altered, neither organic acid nor free HCl is present; the acidity is due to acid salts and combined HCl. If the paper be turned blue, both free HCl and lactic acid may be present. If the red be changed to a dirty brown, lactic acid is probably present without free HCl.

To confirm the presence of free HCl, **test with Gunsberg's reagent** as follows: Boil some water in the saucepan, as a water-bath. Put 3 drops of Gunsberg's fluid and 3 drops of the vomit filtrate into a small evaporating dish; float the latter on the boiling water. Free HCl is indicated by a beautiful rose-red colour appearing, first at the drying margins, and later all over the evaporated residue. The test is easy, but is liable to fail if the vomit be not strained, if more than a few drops of either vomit or reagent be used, or if the water-bath be dispensed with and direct heat employed.

Gunsberg's test gives a similar rose-red colour with any free inorganic acid, such as nitric or sulphuric, also with *strong* solutions of organic acids. There is never

sufficient organic acid in the vomit to do this, nor are nitric or sulphuric acids present: hence Gunsberg's is one of the best for free HCl in the stomach contents.

Next use **Uffelmann's Test for Lactic Acid**. Fill 1 inch of a test-tube with the reagent, add strained vomit drop by drop until the amethyst blue colour is discharged. Free HCl makes the solution colourless, lactic acid a pale canary yellow. It is the *solution* that is yellow. Frequently, on adding acid vomit, a yellowish cloud of phosphates is formed; an alkaline vomit gives a pale orange colour due to a precipitate; in all cases *filter* the mixture. If the *filtrate* is clear pale yellow, lactic acid is present; if a faint yellow, there is a trace; if colourless, none.

The additional tests mentioned above are performed as follows:

Boas's reagent is used exactly as Gunsberg's. A similar, but fainter, rose-red indicates free HCl. Longer heating is necessary, the rose colour sometimes not appearing for ten minutes after evaporation is complete. Allowing for this, Boas's test is almost as satisfactory as Gunsberg's, is cheaper, and the solution keeps good longer.

The Tropæolin Test.—Place a drop of the Tropæolin OO upon a porcelain slab, spread it out in a thin layer, and dry it at 40° C. To do this place the slab close to the foot of the Bunsen burner. When dry, put a single drop of the strained vomit upon the centre of the yellow stain, and continue warming at the foot of the burner as before. A beautiful violet colour appears if free HCl be present. A similar violet is given by *strong* lactic acid, but not with the

amount present in a vomit. The ease of this test strongly recommends itself in private practice.

The Methyl Violet Test is also very simple. Put 1 drop of methyl violet solution upon a white dish close to 1 drop of the strained vomit. A change from violet to bright greenish-blue at their point of coalescence is regarded by many as proof that free HCl is present. There are, however, conditions in which sufficient lactic acid may be present to produce a similar change.

Summary.—With blue litmus-paper, a red reaction indicates one or more of the following: Acid salts, combined HCl, lactic acid, free HCl.

With Congo red, a blue reaction indicates lactic acid or free HCl. A brown reaction indicates lactic acid, and probably no free HCl. With acid salts or combined HCl, the red does not change.

With Gunsberg's reagent, a rose-red colour indicates free HCl.

With Uffelmann's reagent, a yellow colour of the *filtrate* indicates lactic acid.

Boas's reagent (rose red)
Tropæolin OO (violet)
Methyl violet (to greenish-blue)
} May be used as alternatives to Gunsberg's test.

Importance of the Tests.—The absence of free HCl from vomit within an hour of proteid food is physiological. Its absence later indicates deficient acid secretion, resulting in interference with peptic activity and a liability to the growth of organisms. Knowledge of the deficiency is an important indication for treatment, the result of which may be gauged by subsequent testings.

In gastric carcinoma free HCl is often absent, a fact to which diagnostic value has been attached; but it may be absent in many other conditions—for example, in fevers, chronic heart disease, Bright's disease, phthisis, the cachexia of malignant disease elsewhere than in the stomach, cirrhosis of the liver, and, indeed, in almost any debilitating malady.

Presence of lactic acid is not pathological for the first hour after food; longer than this its presence in quantity indicates undue fermentation.

To Estimate the Acidity of the Vomit.

The Total Acidity.—Fill the burette with decinormal soda solution. Pipette 100 c.c. of strained vomit into a beaker. Add 10 drops of phenolphthaleïn solution. Place the beaker upon a white slab under the burette. Add the soda, a few drops at a time, until a definite pink colour appears and persists. It is well to have a second beaker containing colourless acid solution for comparison. The volume of soda solution added gives the amount of caustic soda needed to neutralize the total acids in the 100 c.c. of vomit.

The Acidity due to Free Hydrochloric Acid.—Most methods are too complicated for practical use. The following is simple, and gives approximate results: Prepare a series of small evaporating basins, and into each put 2 drops of Gunsberg's reagent. Pipette 100 c.c. of strained vomit into a beaker. Add decinormal caustic soda from the burette; after the addition of 5 c.c., put 2 drops of the beaker contents into the first evaporating basin, and dry the latter on the

water-bath. Add 5 c.c. more soda to the beaker, put 2 drops of the beaker contents into the second basin, and dry it on the water-bath. Repeat this operation with successive evaporating basins after each addition of 5 c.c. decinormal soda. Suppose a deep rose-red results in the first basin, a less deep with the second, a faint with the third, and none at all with the fourth, it follows that between 15 c.c. and 20 c.c. of decinormal soda are needed to neutralize the free HCl in 100 c.c. of vomit. Now pipette 100 c.c. of strained vomit into a fresh beaker; prepare the basins with Gunsberg's reagent as before; add 16 c.c. of soda to the beaker, and take 2 drops from the latter for the first basin, and similarly 2 drops for successive basins after 17 c.c., 18. c.c., and 19 c.c. of soda have been added respectively. If the rose-red reaction be faint in the first basin, fainter in the second, just perceptible in the third, and absent in the fourth, it follows that between 18 c.c. and 19 c.c., or 18·5 c.c., of decinormal soda solution just neutralize the free HCl in 100 c.c. of vomit.

If the total acidity be already estimated, the difference between the two gives the acidity due to lactic acid, combined HCl, and acid salts.

Importance of the Estimation.—It is unusual in health to find more than 0·2 per cent. of free HCl. More indicates hyperacidity, which may be corrected by prescribing more proteid food to combine with the HCl, or by giving alkalies to neutralize the acid at that period after a meal when it is present in excess.

When doubt exists in diagnosis between gastric carcinoma and a simple ulcer, hyperacidity favours the latter.

To Test for Pepsin.

Into each of four test-tubes put 5 c.c. of strained vomit and 1 disc of egg-white. To the second add 2 grains of dried pepsin, to the third 2 drops of dilute hydrochloric acid (B.P.), to the fourth 2 drops of dilute hydrochloric acid and 2 grains of pepsin. Warm the saucepan water-bath to 40° C., stand all the test-tubes in it, and leave them at this temperature for two hours. The egg in the fourth tube will have swollen up and been partly dissolved. If it be dissolving in the third, and not in the first, acid is deficient in the vomit, but pepsin is present. If it be dissolving in the second, and not in the first, pepsin is deficient. If it dissolve in the fourth, but not in the first three, both pepsin and acid are deficient.

To Test for Rennin.

Neutralize 10 c.c. of strained vomit by adding 2 drops of phenolphthaleïn as an indicator, and dilute caustic soda until a faint pink colour appears. Add 10 c.c. of fresh cow's milk previously boiled and allowed to cool. If rennin be present, the caseinogen of the milk will be precipitated in flakes.

B. MICROSCOPICAL EXAMINATION.

Mix a little of the deposit from the muslin filter with a drop of salt solution upon a slide, put on a cover-slip, and examine it with a low power, shutting off a good deal of the light with the diaphragm.

Particles of food will be seen. **Fat droplets**, highly refractile, structureless globules, large and

small, staining black with a 1 per cent. solution of osmic acid or bright red with Sudan III. **Starch grains**, round or oval, concentrically striated, staining blue with dilute iodine. **Elastic fibres**, double contoured, sometimes branched and twisted spirally at their free ends. **Muscle fibres**, transversely striated, unmistakable. **Vegetable fibres**, also unmistakable.

Importance.—Starch grains, elastic fibres, muscle fibres, may be found when no food containing any of these has been eaten the same day. They then indicate delayed emptying of the stomach.

Vegetable Parasites.—For these the $\frac{1}{6}$-inch power is needed. Two sorts may be looked for:

Sarcinæ ventriculi, or 'packet cocci,' each resembling a bale of wool, tightly corded so as to bulge

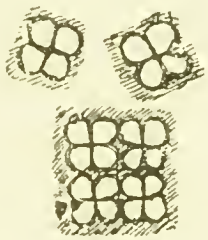

FIG. 71.—Sarcinæ Ventriculi.

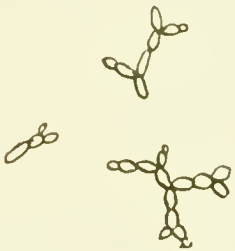

FIG. 72.—Yeast Cells.

between the cords. There may be four cocci in each packet, or any multiple of four (Fig. 71). Each coccus is about the size of a leucocyte, has a yellow tinge, and with iodine stains mahogany-brown.

Yeast fungi, or torulæ, round or oval cells in branching chains or clusters (Fig. 72).

Importance.—Neither is present in health. They indicate chronic delayed emptying of the stomach,

particularly in cases of dilated stomach, whatever the cause.

Test Meals.

Carbohydrate stimulates the flow of gastric juice less than does proteid, but leads to an earlier appearance of free hydrochloric acid. The more proteid given, the less early is free hydrochloric acid to be expected (see p. 122). Before interpreting the results of an examination, it is essential to know what food has been taken, and at what interval before the stomach contents were obtained. In such cases as require particular investigation, therefore, it is usual to give a 'test meal' and to recover it by means of an emetic or by the stomach-tube. The test meals recommended are:

Ewald's Breakfast.

White bread	70 grammes
Weak tea	300 c.c.

Empty the stomach in one hour.

Klemperer's Breakfast.

White bread	70 grammes
Milk	500 c.c.

Empty the stomach in two hours.

Germain See's Lunch.

White bread	150 grammes
Minced meat	80 ,,
Cold water	300 c.c.

Empty the stomach in two hours.

Riegel's Dinner.

Soup	100 c.c.
Beef-steak	60 grammes
White bread	50 ,,

Empty the stomach in five hours.

In practice, Ewald's breakfast is the most convenient.

CHAPTER VI

EXAMINATION OF THE FÆCES

A. GENERAL EXAMINATION.

The fæces are extremely variable in amount, form, colour, and odour; but the following types are never healthy:

The 'Pea-soup' Stool.—Copious, unformed, dirty yellow, very ill-smelling. Chiefly seen in typhoid fever, but by no means always present in this disease.

The 'Rice-water' Stool.—Colourless, odourless, alkaline, watery, with numbers of small white flocculi of epithelium and mucus floating in it. It is seen in cholera, but very similar stools may occur in severe arsenical poisoning and in the very severe diarrhœa that occasionally results from cathartic drugs.

The Blood and Mucus Stool.—Small in amount, without fæcal colour, often odourless, consisting of slimy mucus mixed with bright-red blood. It occurs in several conditions, notably intussusception, acute colitis, ulcerative colitis, dysentery.

The Clay-coloured Stool.—May be well or ill formed; usually offensive, but not always so. The colour resembles that of putty, and is due to absence of bile pigments. It indicates obstruction of the large bile-ducts, as by catarrh, gall-stone, or growth. It is

therefore associated with jaundice; but jaundice may occur without clay-coloured stools when the large bile-ducts are free, as in cirrhosis of the liver.

The Fatty, Clay-coloured Stool.—When bile is absent fat digestion is imperfect, but when neither bile nor pancreatic juice reach the bowel it almost ceases. A copious iridescent scum of fat upon a clay-coloured motion suggests occlusion of both bile and pancreatic ducts, such as results from carcinoma of the head of the pancreas, or from chronic pancreatitis.

The Infantile Zymotic Enteritis Stool.—Very scanty, drying upon the diaper as a grass-green stain of a most offensive odour; occasionally streaked with blood.

The Purulent Stool.—Obvious pus occurs sometimes, coating the motion, or coming from the bowel as a separate evacuation. It may occur in dysentery or ulcerative colitis, but more often follows rupture of an appendicular or other abscess into the colon or rectum.

The Mucous Colitis Stool.—Consists of mucus in the form of white gelatinous casts of the interior of the bowel. Under the microscope the casts are transparent and structureless; embedded in them are fragments of undigested food, leucocytes, scattered epithelial cells, blood-corpuscles, micro-organisms, and crystals of phosphates and of cholesterin. They have every variety of length, from an inch up to some feet; are often narrow and riband-like, suggesting tape-worms, but distinguished at once by their not being segmented. They are diagnostic of mucous

colitis, a most troublesome disorder, occurring in women of nervous temperament. It is common for such patients to pass *intestinal sand* also from time to time. The 'sand' may amount to a drachm or more at an evacuation. It consists of small granules of inorganic salts, especially phosphate, carbonate, and sulphate of calcium, together with a certain amount of organic matter. The sand often has a reddish-brown or a green colour.

The Stool with Blood in it.—The character of blood in a motion varies with the site of the bleeding-point. Bright streaks of blood upon the outside of a stool indicate hæmorrhage near the rectum—*e.g.*, from piles, or sigmoid or rectal carcinoma or other stricture. Bright blood mixed with the fæces comes from higher up—*e.g.*, from tuberculous, typhoid, or other ulceration of the ileum or cæcum. Blood and mucus occur with intussusception or inflammation or ulceration of the colon. When bleeding is high up—*e.g.*, from a gastric or duodenal ulcer, or from ruptured varicose veins at the lower end of the œsophagus in cases of cirrhosis of the liver—or when blood has been swallowed and passed on, the motions are black and tarry, a condition called melæna. Black motions may also be due to—

1. Certain drugs.

Iron. Manganese. Bismuth.	Converted by the sulphuretted hydrogen in the bowel into sulphides.

2. Certain foods, notably bilberries and charcoal biscuits.

To confirm the presence of blood, a little of the

motion may be smeared upon a piece of lint, a drop of tincture of guaiacum placed by the side of it, and ozonic ether poured over both. If blood be present, a bright blue colour appears. The guaiacum reaction, however, has many fallacies (p. 27), and a more conclusive test is with the spectroscope. Shake up a little of the fæces with water in a test-tube, allow the solid particles to settle, and examine the supernatant fluid for the absorption bands of hæmoglobin (p. 29).

The Stool with undigested Milk in it.—The curds may be obvious to the naked eye. Whenever patients who are upon milk diet suffer from diarrhœa —*e.g.*, in typhoid fever—it is important to examine the fæces before ordering astringents. Should curds be present, modification of the milk with lime or barley-water may cure the diarrhœa at once.

B. SPECIAL EXAMINATIONS.

Gall-stones.—Mix the fæces with water, and pour them on to a fine meshed sieve beneath a running tap; triturate well with a stick. A gall-stone, if present, will become visible after the softer fæcal matter has passed through. It may be an obvious gall-stone to the naked eye; if there is any doubt, test it as follows:

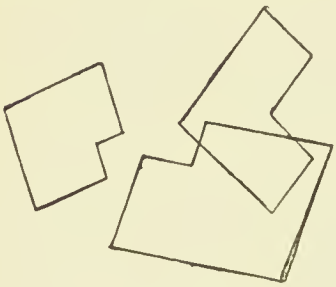

Fig. 73.—Cholesterin Crystals.

1. **For Cholesterin.**—Dissolve a portion of the stone in chloroform; add sulphuric acid. If choles-

terin be present, the solution assumes a blood-red colour, going on to purple.

Or, extract a part of the stone with ether; evaporate the solution; dissolve the residue in absolute alcohol, and allow to crystallize. Examine for the characteristic crystals of cholesterin (Fig. 73) under the low power.

2. **For Bile Pigments.**—Dissolve a portion of the stone in weak hydrochloric acid. Apply Gmelin's test (p. 38) to the solution.

Importance.—Discovery of a stone in the fæces after an attack of biliary colic is the best proof that the stone has been got rid of. Should the stone be facetted, other gall-stones are, or have been, present.

Typhoid Sloughs.—These may be looked for on a sieve in the same way as gall-stones.

Importance.—Less now than formerly. Previous to the discovery of the Widal test, the diagnosis of typhoid fever often depended on finding sloughs from Peyer's patches in the stools.

Bacteria.—These swarm in fæces, and are for the most part unimportant. To examine them, pick out a very small portion of the excretion with forceps, make and fix a film, and stain by the methods described on p. 108, *et seq.* The organism of importance are—

The Cholera Vibrio (p. 116).

The Amœbæ of Dysentery.—These can only be found in stools less than twelve hours old; later they disintegrate. They are best obtained from the smaller pellets of mucus. Each is a rounded or irregular protoplasmic mass about 30 μ in diameter, finely granular, refractile, with several vacuoles, and ingested

red corpuscles or bacteria (Fig. 74). In warm 0·6 per cent. salt solution they may be watched sending out blunt pseudopodia, and gradually crawling along the slide. In the fresh state they show no nucleus, but stained specimens show one about the size of a red corpuscle. The amœbæ disintegrate with heat, so that films cannot be fixed in the ordinary way; they should be immersed for five minutes in the following solution :

Gulland's Fixing Solution.

Absolute alcohol	25	c.c.
Pure ether	25	,,
Solution of corrosive sublimate in absolute alcohol (strength, 2 grammes in 10 c.c.)	0·5	,,

and then stained with carbol methylene blue or with carbol fuchsine (p. 108).

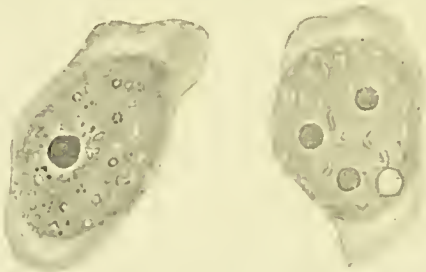

FIG. 74.—Amœbæ of Dysentery.

The amœbic form of dysentery is only one of several, but it is the kind most liable to be followed by hepatic abscess.

The **Typhoid Bacillus** is so similar to the *Bacillus coli communis* (p. 112) that the two cannot be distinguished without elaborate cultural methods.

Intestinal Parasites.

These are of two classes: I. Cestodes, or tape-worms. II. Nematodes, or thread-worms.

I. **Cestodes.**—These have two life cycles: a sexual, the hermaphrodite tape-worm, in one host; and an asexual, cystic, in another. There are three whose tape-worm stage occurs in the human small intestine—namely:

Tænia Solium.—Its intermediate host is the pig; the head of the worm (Fig. 75) has four round suck-

FIG. 75.—Head of Tænia Solium.

FIG. 76.—Head of Tænia Mediocanellata.

FIG. 77.—Head of Bothriocephalus Latus.

ing-discs and a single row of hooklets, about twenty-six in number.

Tænia Mediocanellata.—Its intermediate host is the ox; the head of the worm (Fig. 76) has four round sucking-discs, and no hooklets.

Bothriocephalus Latus.—Its intermediate host is a fish, such as the pike; its head (Fig. 77) has two longitudinal sucking-discs, and no hooklets.

These worms vary from 10 to 20 feet in length, and are composed of hundreds of opaque white segments

joined end to end. Each segment is a complete hermaphrodite organism, containing an ovary, a testis, and a water-channel system. At the upper end of the worm is a narrow neck, and a head not much larger than that of an ordinary pin. After the administration of anthelmintics, diligent search should be made for this head, for there is no certainty of the eradication of the worm until the head itself has been passed. It is by the characteristics of the head that the variety of tape-worm is diagnosed.

The head of *Tænia echinococcus* has four round sucking-discs like that of *Tænia solium*, but forty hooklets in two rows instead of twenty-six hooklets in one. The worm, $\frac{1}{4}$ inch long, consists of a head, a neck, an immature and a mature segment; it inhabits the intestine of the dog, and it is only the cystic stage which affects man, causing hydatids.

II. **Nematodes.**—These have the sexes distinct.

Ascaris Lumbricoides.—This is 6 or 8 inches long; is not unlike an earth-worm, but is distinguished by the following points: (1) There is no imbrication of segments, so that the finger can be drawn along it either way without catching; (2) the mouth is at the extremity instead of under a blunt prostoma, as in the earth-worm; (3) it tapers towards each end; (4) it has no cingulum or fleshy band, such as surrounds the earth-worm at the junction of the anterior and middle thirds.

Oxyuris Vermicularis.—This is $\frac{1}{3}$ inch long, blunt at the head end, tapering to a pointed tail (Fig. 78), and appearing in the fæces like white, slowly moving

short threads. It inhabits the large intestine, and is common in children. Enormous numbers may be passed by the same patient.

FIG. 78.—Oxyuris Vermicularis.

Ankylostomum Duodenale.—This inhabits the duodenum. Not uncommon in the East, it is rare in

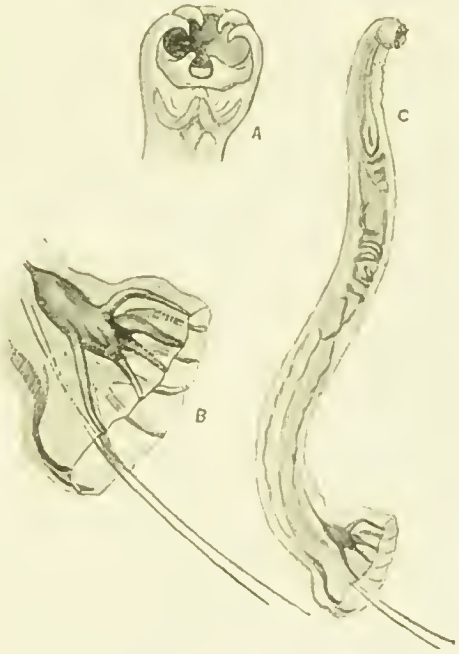

FIG. 79.—Ankylostomum Duodenale.
A. Much-magnified head.
B. Much-magnified tail of male.
C. Entire parasite, less magnified.

England, though occasionally there are epidemics like that amongst the Cornish miners. Should ankylos-

tomiasis be suspected, thymol should be administered, and the fæces examined for the worms. Each is ¼ inch long, greyish-white in colour, cylindrical; the posterior end is broad, and in the male is provided with an umbrella-like expansion with eleven ribs. The worm tapers forwards to a narrow neck, which ends in a bulging mouth, the margin of which is furnished with four characteristic claw-like hooks and two blunt teeth (Fig. 79), which enable the parasite to adhere firmly to the duodenal wall. The symptoms of ankylostomiasis are so similar to those of pernicious anæmia that cases are easily overlooked. Eosinophilia may suggest the diagnosis, which is confirmed by giving thymol and finding the parasite in the fæces.

Trichocephalus Dispar.—This is about 1½ inches long, and consists of an opaque white body often wound up like a watch-spring, and a long thread-like anterior extremity (Fig. 80). It inhabits the cæcum.

Fig. 80.—Trichocephalus Dispar.

Though not uncommon, it causes no symptoms. It is acquired by ingestion of food containing ova derived from the dejecta of other persons who have the worm.

The Ova of Intestinal Parasites.—These, when abundant, may be found in simple smears of the fæces. More often it is necessary to concentrate them. The following is an easy way of doing this: Put as much fæces as would lie on a shilling into a test-tube; fill the latter three-parts full of normal salt solution (0·6 per cent.). Cork it, and shake the

contents violently for some seconds. Now allow the tube to stand for half an hour. The heavier portions of the fæces, including the ova, will have subsided to the bottom. Pour off the dirty supernatant liquid, and replace it with fresh normal saline. Shake violently again, to disintegrate as many of the larger fæcal fragments as possible. Allow to stand, and repeat the process until the normal saline becomes practically clear after standing for half an hour. Now pour off the surface fluid, transfer a drop of the sediment to a slide with a pipette, cover the drop, and examine the preparation under a medium power, using a mechanical stage so as to ensure every part being looked at.

A kaleidoscopic conglomeration of all sorts of fragments and cells will be seen, amongst which striated muscle fibres, connective tissue, epithelial cells, leucocytes, spiral vessels from plants, vegetable cells, carbonaceous particles, phosphatic crystals, hair stumps, pieces of keratin, bacteria, and debris will occur. Ova, if present, attract attention at once. Before experience is gained, all sorts of things may be taken for them, but once seen they cannot be mistaken. They are all of comparatively large size, of perfectly even outline, with a definite capsule surrounding an indefinite embryo, and they are mostly stained a deep brown by the fæcal pigment stercobilin. They can easily be seen with the low power, and they are quite large under the $\frac{1}{6}$-inch objective.

The most characteristic of all the ova is that of *Trichocephalus dispar* (Fig. 81), each being an elongated oval with clear rounded bulges at the ends. The eggs

of the *Tænia solium* and *T. mediocanellata* are much alike, each being almost round, with a very thick envelope marked with highly characteristic radiating lines (Fig. 82). *Oxyuris vermicularis* ova never occur in

FIG. 81.—Ovum of Trichocephalus Dispar.

FIG. 82.—Ovum of Tænia Solium.

Both are magnified to the size as seen under $\frac{1}{6}$ inch objective.

the stools; the worm leaves the body to lay them. The egg of *Ascaris lumbricoides* (Fig. 83) is a broad oval, and consists of unsegmented protoplasm with a clear, thick capsule, and a characteristic brown uneven

FIG. 83.—Ovum of Ascaris Lumbricoides.

Slightly more magnified than are Figs. 81 and 82.

envelope outside this. *Bothriocephalus latus* ova have no radiating lines in the capsule like those of *tæniæ*. If the eggs of *Ascaris lumbricoides* had the outermost envelope removed, and the internal protoplasm coarsely segmented into a mulberry-like mass, it would be like the egg of *Bothriocephalus latus*.

CHAPTER VII

MICROSCOPICAL EXAMINATION IN AFFECTIONS OF THE SKIN

Ringworm.—Though more than two varieties have been described, it is sufficient for practical purposes to make the following two classes :

1. **Tinea tonsurans**, ringworm of the scalp, due to a vegetable parasite, which forms a mycelium inside the hairs, produces small spores, and is called the **Trichophyton microsporon endothrix.**

2. **Tinea circinata**, ringworm of the body, due to a vegetable parasite, which forms a mycelium outside the hairs, produces large spores, and is called the **Trichophyton megalosporon ectothrix.**

There is a third parasite described, with large spores and its mycelium inside the hair—the **Trichophyton megalosporon endothrix.** Apparently, this is much rarer than the other two.

The essential things to look for are the mycelium and the spores. With forceps pick out one of the stubbly hairs from the suspected patch, and proceed by one or other of the following methods :

(*a*) **To Detect the Mycelium.**—Immerse the hair in ether for fifteen minutes, or for twenty-four hours

if there be time, in order to remove the grease; transfer to liquor potassæ for fifteen minutes, wash in water, and place in a drop of glycerine upon a slide apply a cover-slip; press it down; blot up the excess of glycerine; ring the specimen with Canada balsam, and examine under the $\frac{2}{3}$-inch objective. The mycelium may be seen within or around the hair.

(*b*) **To Stain the Spores.**—Immerse the hair in ether to remove the fat as before; then stain by Gram's method (p. 108), and mount in glycerine; or dehydrate with absolute alcohol, clear with xylol, and mount in Canada balsam. Examine under the $\frac{1}{6}$-inch power. The spores will be deep violet, and whether

FIG. 84.—Spores on Hair in Tinea Tonsurans.

FIG. 85.—Spores on Hair in Tinea Circinata.

the mycelium be within the hair or upon the outside, they are for the most part on the surface. In the case of tinea tonsurans the small spores form a closely packed casing to the hair, resembling a mosaic (Fig. 84). In tinea circinata, on the other hand, the large spores are less densely packed, and form rows which have been compared to rosaries (Fig. 85).

Tinea Versicolor.—With the back of a scalpel

scrape the surface of one of the brown patches of affected skin; transfer the fine scrapings to a drop of liquor potassæ on a slide; apply a cover-slip, and examine under $\frac{1}{6}$-inch objective. Amongst clumps of epithelial debris will be seen the **Microsporon furfur**, interlacing jointed threads of colourless mycelium with bunches of spores between them.

Favus.—Pick out a hair and as small a portion of the surrounding crust as possible. Transfer it to a drop of liquor potassæ on a slide, apply a cover-glass, press it down, and examine with a $\frac{2}{3}$-inch objective. A fungus, the **Achorion Schonleinii**, will be seen as a dense branching mycelium, completely infiltrating the hair, and forming a dense coating of large spores upon its surface. It closely resembles a trichophyton. The affection is rare in England. A typical case is easy of recognition, but if the primary affection be obscured by impetigo, diagnosis without the microscope may be difficult.

Scabies. — The parasite is called **Sarcoptes hominis**, or **Acarus scabiei**, and it is related to the spiders. There are two sexes. The male lives upon the surface of the skin, is the smaller, and is very difficult to find. The female, when impregnated, penetrates the stratum corneum, and burrows along in the rete Malpighii, depositing eggs as she goes. Her point of entry is marked by a vesicle, and her path by a typical black 'run.' To obtain the parasite, lay a pin flat upon the surface of the skin, and push it with a rotatory movement into the epidermis at that end of the burrow which is away from the vesicle, taking care not to draw blood. The acarus appears

as a small pearly object at the end of the pin. Under the microscope it is oval, with eight legs, two in front and two behind on each side, with a mouth between

FIG. 86.—Ventral View of Female Sarcoptes Hominis.

the front legs, and a few short hairs at the tail end (Fig. 86).

CHAPTER VIII

EXAMINATION OF SEROUS EXUDATIONS, CEREBRO-SPINAL AND CYSTIC FLUIDS

Serous Fluids from the Peritoneal or Pleural Cavities.—These are alkaline in reaction, contain 1 per cent. of salts, are usually of a straw-yellow colour, and are either quite clear or slightly turbid. The points to examine are—

The Specific Gravity.—This may be taken in a specimen-glass with an ordinary urinometer. It varies from 1005 to 1035.

The Amount of Albumin.—This may be estimated by Esbach's albuminometer, the fluid being suitably diluted with water if necessary (p. 48). It varies from 2 to 40, but more commonly lies between 10 and 30, parts per 1000.

Importance.—From the specific gravity of the fluid and the amount of albumin it contains it is sometimes possible to decide whether an effusion is inflammatory, or whether it is a passive dropsy such as occurs with cardiac failure. The specific gravity and the amount of albumin are higher in the former than in the latter. Definite conclusions, however, can only be drawn when

the figures are extreme: that is to say, a specific gravity of 1020 or more, or 30 parts per 1,000 of albumin, would indicate an inflammatory origin; a specific gravity of 1010 or less, or 5 parts per 1,000 of albumin, would favour a diagnosis of passive dropsy; figures between these would be compatible with either.

The specific gravity is no measure of the amount of albumin present. Ascitic fluid of low specific gravity may contain much albumin.

The Presence or Absence of Blood.—This may be looked for by the guaiacum test (p. 27), or by the microscope or spectroscope (p. 29).

Importance.—It is common to find blood in the later portions of a tapping, or in a serous effusion that has been tapped before; but if the fluid contain blood at the first tapping, it either indicates a very acute inflammation, or rouses a suspicion of malignant disease.

Cell Elements.—These are best examined by allowing the fluid to stand an hour or more in a specimen-glass, pipetting off and centrifugalizing the lowest part, preparing and staining a film of the deposit, and examining it under the high power. There may be no cells at all, or there may be leucocytes, squamous pleural or peritoneal cells, red corpuscles, or even particles of malignant growth. It is rare to find bacteria, owing to the great bulk of fluid in which they are suspended.

Importance.—The presence of many cells, particularly leucocytes, is almost proof of the inflammatory origin of the effusion. It is said that preponderance of lymphocytes indicates tubercle.

Spontaneous Coagulation.—This may occur in

both inflammatory and in passive effusions on standing. Should the floating jelly-like coagulum be large and appear quickly, the fluid is probably inflammatory, but otherwise no deduction can be drawn.

The Presence of Fat Globules.—In rare cases the fluid is almost like milk, and the microscope reveals droplets which stain black with osmic acid, or red with Sudan III. (p. 92). When the fluid comes from the peritoneum the condition is termed *chylous ascites*, of which one chief cause is infection with filaria. It may also result from rupture of the receptaculum chyli after abdominal injury or obstruction to the thoracic duct. Chylous effusion in the chest is very rare, but has occurred after injury to the thoracic duct in the neck.

Cerebro-spinal Fluid.—This is clear, colourless, alkaline, with a specific gravity which may be as low as 1002. It contains 1 per cent. of salts, a mere trace of albumin, and a body—pyrocatechin—which reduces Fehling's solution on boiling, but does not ferment with yeast. After head injury, escape from the ear or nose of a clear fluid which reduces Fehling's solution is strong evidence that the base of the skull has been fractured.

Pancreatic Cyst Fluid.—This may be either clear or turbid. It is of watery consistence, alkaline, with 1 per cent. of salts, a specific gravity of about 1005, and little or no coagulable proteid. The essential characteristic is that it contains proteolytic and amylolytic ferments. These may be tested for as follows:

1. **Proteolytic Ferment.**—Put a disc of hard-boiled white of egg into a test-tube, add some of the fluid, and stand in a water-bath at 37° C. for an hour.

Should pancreatic ferment be present, the egg-white will have become partially digested.

2. **Amylolytic Ferment.**—Boil some water in a test-tube, and carefully stir in some finely powdered starch until a thin mucilage is obtained. Cool to 37° C., and add some of the fluid. From time to time take a drop of the liquid and test it with a drop of iodine. If amylolytic ferment be present, the starch will rapidly cease to go blue with iodine, but will presently go red instead, and, finally, will remain colourless, from the formation, in the first instance, of erythrodextrin, and later of achroödextrin. The ferment will ultimately convert the latter into dextrose, which may be detected by its reduction of Fehling's solution.

Importance.—The exact nature of a cyst in the upper part of the abdomen may be obscure even after laparotomy. Its pancreatic origin is proved beyond doubt if the above ferments are detected in the fluid from it.

Hydatid Cyst Fluid.—This is colourless, clear or opalescent, alkaline, and has a specific gravity of about 1010. It contains but a trace of albumin, and by this may be distinguished from ascitic fluid. The main diagnostic point, however, is the presence in it of the 'hooklets' (Fig. 87) of dead scolices. It must be borne in mind that a hydatid cyst may grow to a great size, with production of daughter and granddaughter cysts, and yet may never have produced scolices. The fluid from such a 'barren' cyst will contain no hooklets.

Fig. 87.—Hydatid Hooklets.

Hydrocele Fluid.—Its characters are similar to

those of ascitic fluid, and almost as variable. A spermatocele is distinguished from a hydrocele by the presence of spermatozoa in the former. They may be detected in a drop of the centrifugalized deposit from the fluid (Fig. 17, p. 19). It is not uncommon to find cholesterin crystals (Fig. 73, p. 134) in hydrocele and spermatocele fluids.

Ovarian Cyst Fluids.—There are two types:

1. Ovarian cysts contain a viscous brownish-green fluid, which pours almost like treacle. It has a high specific gravity (*e.g.*, 1030), alkaline reaction, and 1 per cent. of salts. Albumin is present in variable quantity; the viscosity is due to a substance called **pseudo-mucin** or **metalbumin**. It differs from mucin in not being precipitated by acetic acid, and from albumin in not being coagulable by heat. On the addition of alcohol it forms a dense stringy precipitate, and after boiling with HCl it yields a substance which readily reduces Fehling's solution. Microscopical examination of the deposit usually shows much detritus, along with red corpuscles and leucocytes, large epithelial cells, which are sometimes in clumps, cholesterin crystals, and large circular, highly refractile colloid granules, which are said to be characteristic.

2. Parovarian cysts contain watery, colourless fluid, alkaline, of low specific gravity (*e.g.*, 1006), with 1 per cent. of salts. Albumin is scanty, pseudo-mucin is absent, and cells are very few.

Importance.—The nature of the fluid may be the only means of determining in what structure the cyst arose. The prognosis is more serious in the case of an ovarian than in that of a parovarian cyst.

CHAPTER IX

THE PRESERVATION AND STAINING OF MORBID ANATOMY SPECIMENS

MORBID anatomy specimens may be preserved in one or other of three ways, namely :

I. In bulk, without regard to keeping the natural colour.

II. In bulk, with retention of the natural colour.

III. For microscopical purposes.

I. **Preservation in Bulk without Colour.**—The simplest method is to use either (*a*) rectified spirit, or (*b*) 5 per cent. solution of formaline in ordinary water. Care should be taken that the shape of the tissue is correct before it is immersed in the fixing fluid; cavities should be gently stuffed out with cotton-wool, and there should be wool in the bottom of the vessel to prevent deformity from the weight of the tissue. Much fluid should be used to start with, and it should be changed once or twice subsequently. The formaline penetrates more rapidly than the spirit. Both cause shrinkage of cells, so that if microscopical sections are to be cut it is well to fix a small portion specially for this purpose (p. 153). Hardening will be complete in a week or two. The specimen can then be kept in

just as much of the fluid as will cover it, sealed up in a suitable wide-necked jar.

II. **Preservation of Tissues for Colour.**—The colour of most tissues is due to hæmoglobin or its derivatives, to preserve which special methods are required. **Kaiserling's method** is one of the best. The three following solutions are needed :

1.	Pure formaline	1,600 c.c.
	Potassium acetate	17 grammes
	Potassium nitrate	90 ,,
	Glycerine	300 c.c.
	Water	8,000 ,,
2.	Alcohol	90 per cent. solution.
3.	Distilled water	9,000 c.c.
	Potassium acetate	1,188 grammes
	Filter, and then add glycerine	3,000 c.c.

The tissue should not be exposed either to the air or to any moisture longer than can be avoided before it is immersed in a large bulk of the first fluid, which should be changed next day, and the tissue left in it for about a week. It is then of a dull dark colour. Transfer it to the 90 per cent. alcohol, and leave it until the natural colour of the tissue reappears—this should be in from one to six hours. Transfer it to the third fluid, in which, after one or more changes, as may be necessary, it is finally mounted. It is well to keep the preparation unexposed to any bright light.

III. **Hardening and Fixing for Microscopical Purposes.**—Spirit causes so much shrinking and deformity of the cells that it is unwise to use it. For most ordinary purposes the best fixing fluid is a saturated solution of **corrosive sublimate** :

Mercuric chloride	6·5 grammes
Water	100 c.c.

If normal saline (0·6 per cent.) be used instead of plain water, rather more mercuric chloride is required for saturation, and the results are slightly better. A piece of tissue, about $\frac{1}{2}$ inch in diameter and $\frac{1}{3}$ inch thick, should be immersed in this for twenty-four hours; if left longer it becomes too hard and brittle. It should now be soaked in water for twelve hours, and afterwards carried through a series of bottles containing successive strengths of alcohol—25 per cent., 50 per cent., 75 per cent., 90 per cent., and absolute—being left for twelve hours in each before transference to the next. After twelve hours in the last, it should be put into a second bottle of absolute alcohol for another twelve hours, to ensure absolute dehydration.

Embedding in Paraffin.—For actual embedding and section the tissue will usually be sent to a special laboratory. This is best done at the 75 per cent. alcohol stage, at which the tissue can be kept indefinitely. The process of embedding in paraffin is, briefly, as follows :

From absolute alcohol the tissue is put into three changes of xylol, in each of which it stays twelve hours. If still opaque, it is not quite dehydrated, and should be returned to absolute alcohol. If quite clear, it is taken from the xylol, the excess of which is blotted off, and put into paraffin wax kept just melted on a water-bath. The wax should be such as has a melting-point of 48° C. In twelve hours the tissue is transferred to fresh paraffin, and again into fresh for another twelve hours. The xylol by this time is all evaporated, and its place in the tissue taken by paraffin. A special

mould is now got ready, filled with melted paraffin, and into it, with that surface downwards which it is desired to cut, the tissue is put with a pair of forceps warmed sufficiently to prevent solidification of the paraffin on them. As it cools, the paraffin in the mould sets, a process which is completed by immersing the whole in cold water. The block is then taken from the mould, the excess of paraffin removed with a sharp knife, and the embedded tissue is ready for fixing to the microtome and cutting. One of the best instruments for the purpose is the Cambridge Improved Rocking Microtome.

Mounting and Staining the Sections. — The paraffin sections should be floated out on water which is just warm enough to make them go flat. A microscope slide is partly immersed in the water, and held obliquely under a section, which is then drawn into position by means of a needle. The slide is removed from the water with the section on it, and after the excess of water has drained off, it is placed to dry on a water-bath whose temperature is just less than the melting-point of the paraffin. In about twelve hours it is ready for staining as follows : Pour on a few drops of xylol to dissolve away the paraffin ; remove the xylol with absolute alcohol ; wash in water to remove the alcohol ; pour on a few drops of Gram's iodine solution (p. 109), and leave for a minute or two. The object of this is to remove the last traces of corrosive sublimate by converting it into the more soluble iodide. If the iodine is not used, the final preparation is very apt to contain numbers of nail-like crystals of sublimate ; wash off the iodine with spirit, and immerse the

section in **Ehrlich's acid hæmatoxylin**, the formula of which is as follows:

Hæmatoxylin	2 grammes
Glacial acetic acid	10 c.c.
Absolute alcohol	100 ,,
Glycerine	100 ,,
Water	100 ,,
Alum to saturation.	

The stain is best when it has been made up a month or more. It should be filtered from time to time; and, as it stains very deeply, it is wise to dilute it with twice its bulk of fluid made up as above, but without the hæmatoxylin. After staining for five minutes, wash the preparation well in gently running tap-water. The section, previously red, will be turned blue by the alkali in the latter. When quite blue, immerse for not more than thirty seconds in aqueous eosine (p. 75); remove excess of the latter quickly with spirit; remove the spirit with absolute alcohol to dehydrate the section completely; immerse in xylol, and, if the section does not become transparent throughout, return to absolute alcohol; drain off the excess of xylol, and mount the slide in Canada balsam. Cell nuclei are stained blue, cell protoplasm and connective tissue red.

The Freezing Microtome.—This has the advantage of producing sections quickly, and is used when immediate examination is required—for example, during operations, when it is not certain whether a tumour is malignant or not. The principle is to freeze the tissue in gum by means of an ether spray. Full directions are issued with the instrument.

Embedding in Celloidin.—Some tissues, notably eyes and brain matter, are unsuitable for embedding in paraffin; celloidin is used instead. The tissue is carried through to the absolute alcohol stage, as described on p. 154. Then, instead of xylol, it is put into a mixture of equal parts of absolute alcohol and ether for six hours, and transferred first to a thin and then to a thick solution of celloidin, in each of which it remains a week. It is then mounted in thick celloidin upon the microtome block-carrier, and placed in a desiccator until firm enough not to pit on pressure. The block is further hardened in 80 per cent. spirit for a couple of days, after which sections are cut upon a special microtome.

Müller-Formol Fixing Fluid.—This is used in preference to corrosive sublimate for brain and spinal cord tissues. Its formula is as follows:

Müller's fluid	Sodium sulphate ..	25 grammes
	Potassium bichromate	25 ,,
	Water	1,000 c.c.
	Formaline	50 ,,

The formaline should be added to the Müller's fluid immediately before use. Large pieces of tissue require to be in it for a week or more, during which the fluid should be changed at intervals. Small pieces are fixed in about a day.

Decalcification of Bony Tissues.—It is not possible to cut sections of bony tissues until they have been decalcified. They should first be fixed like other tissues, after which the mineral salts are gradually removed by immersion in repeated changes

of weak nitric or hydrochloric acid. The following is a simple **decalcifying fluid**:

Alcohol, 75 per cent. solution	.. ..	100 c.c.
Non-fuming strong nitric acid	.. ..	2 ,,

The process takes a month or more, after which the tissue is dehydrated and embedded in paraffin.

The Stain for Amyloid (Lardaceous) Tissues.—The paraffin section, after the water stage following the iodine has been reached (p. 155), is immersed for ten minutes in a saturated solution of methyl violet in distilled water. It is then well washed, first in water, then in 1 per cent. acetic acid, and then in water again. It cannot be dehydrated, because spirit removes all the stain. It should therefore be mounted in glycerine, the cover-slip being ringed with Canada balsam. The lardaceous material stains bright pink, the other tissues violet.

CHAPTER X

TESTS FOR THE COMMONER POISONS

Alkaloids.—Before testing for the following alkaloids they must be extracted from the gastric contents. One of the simpler processes for this is as follows:

Otto's Method.—Acidify the vomit with weak sulphuric acid to convert the alkaloid into the sulphate. Strain through muslin. Agitate the filtrate with ether to remove all fat. Pour off the ether; render the residual watery solution alkaline with potassium carbonate to liberate the alkaloid. Shake up with ether again; the sulphate of the alkaloid was insoluble in ether, but the alkaloid itself is soluble. Concentrate the ethereal solution, and test it as follows:

Belladonna.—Add a little fuming nitric acid; dry upon a water-bath; cool, and moisten with a drop of potash dissolved in absolute alcohol. Atropine will give a *violet* colour changing to red.

Opium.—Evaporate the ethereal solution to dryness; dissolve in cold strong sulphuric acid, and add a drop of strong solution of bichromate of potash. Morphia gives a bright *green* colour.

Strychnine.—Evaporate the ethereal solution to dryness; to the residue add 2 drops of sulphuric acid, and then 2 drops of strong bichromate of potash solution. Strychnine will give a beautiful *purple-blue* colour changing to crimson, and finally to a light red.

Arsenic—1. *Marsh's Test.*—Put a piece of pure zinc into a flask (Fig. 88, B), cork, and through the thistle funnel C pour a little distilled water. Add pure sulphuric acid until there is brisk evolution of hydrogen. Collect a little of the gas escaping from the side-tube A in a test-tube, and ignite it. If it burn in the test-tube without explosion all the air has been expelled from B. Ignite the hydrogen jet A. Hold a cold porcelain dish in the flame. No stain should be deposited on the white surface if the hydrogen be pure. Now pour in some of the suspected stomach contents through C, and presently hold the cold porcelain dish in the flame A again. If arsenic be present it will be coming off as arseniuretted hydrogen, and will be deposited as a shining black metallic stain on the white dish.

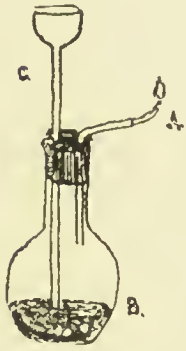

FIG. 88.

Antimony will give a similar black stain, but it lacks the metallic lustre.

2. *Reinsch's Test.*—Strongly acidify the stomach contents with hydrochloric acid, and allow to stand one hour. Filter. Introduce into the filtrate a brightly polished strip of copper foil and boil. If arsenic be present, a steel-grey coating will appear upon the copper. This may be further tested by

dissolving in nitric acid and evaporating to dryness. On adding silver nitrate to the residue a brick-red precipitate of arsenate of silver will appear.

The difficulty of the test is to insure both the acid and the copper being free from arsenic to begin with.

Carbolic Acid.—Just acidify the gastric contents with hydrochloric acid. Filter. To the filtrate add bromine water. Carbolic acid gives a dense yellow precipitate of tribromophenol.

Chloral.—This is not easy of detection, but may be tested for as follows: Shake the suspected fluid up with pure ether, which will dissolve the chloral hydrate; separate off the ethereal solution, add silver nitrate and ammonia, and boil. If chloral is present in any quantity a mirror of metallic silver should form.

Coal-Gas.—The patient's blood will be a bright cherry-red colour. The spectroscope will give the bands of carboxyhæmoglobin identical with those of oxyhæmoglobin (p. 29), but not altering to that of reduced hæmoglobin on adding ammonium sulphide and shaking up well.

Copper Salts.—With a platinum wire hold a little of the suspected fluid in a Bunsen burner or spirit flame. Copper gives a bright green colour to the flame. Add HCl, and evaporate the stomach contents to small bulk. Filter. Add potassium ferrocyanide to the filtrate. Copper will give a characteristic chocolate precipitate.

Corrosive Sublimate.—Mix the gastric contents with an equal bulk of ether, and shake up well. The ether will dissolve the corrosive sublimate. Pour off

the ethereal solution; concentrate it by evaporation; add iodide of potassium. Mercury will give a scarlet precipitate of iodide of mercury, soluble in excess of potassium iodide.

Hydrochloric Acid.—Filter the acid stomach contents. Add nitric acid to the filtrate, and then silver nitrate solution. A dense white precipitate of silver chloride results. Normal gastric juice contains hydrochloric acid, which will give the same test. A control must be made with a 0·2 per cent. hydrochloric acid solution, and the density of the two precipitates compared.

Lead Salts (see p. 45).

Nitric Acid.—Neutralize the gastric contents with carbonate of potash. Filter. Evaporate the filtrate to small bulk. Mix with an equal bulk of strong sulphuric acid; allow to cool *completely*. Pour into a test-tube, and carefully add a solution of ferrous sulphate. The latter fluid floats upon the former, and the appearance of a black ring at the junction of the two indicates nitric acid.

Oxalic Acid.—Boil the gastric contents with caustic potash. Even if lime has already been given to the patient, potassium oxalate will be formed. Filter. To the filtrate add solution of calcium sulphate. Oxalic acid will give a dense white precipitate, insoluble in acetic, but soluble in hydrochloric, acid.

Phosphorus.—Dilute the vomit with twice its bulk of water, acidify with sulphuric acid, transfer to a glass retort with a long condensing tube, and distil in the dark. Minute traces of phosphorus will render the condensing vapour luminous.

Prussic Acid.—This may be evident by the smell. To test for it, exactly neutralize the gastric contents, whether they be acid or alkaline, transfer them to a flask, and distil slowly upon a water-bath. To the distillate add silver nitrate; prussic acid will give a curdy white precipitate, insoluble in cold nitric acid, but soluble in hot. To confirm, add liquor potassæ and hydrochloric acid to the precipitate, and then a few drops of sulphate of iron solution. If prussic acid be present, Prussian blue will be formed.

Sulphuric Acid.—Filter the acid stomach contents. Add a solution of barium chloride to the filtrate. Sulphuric acid gives a dense white precipitate of barium sulphate, insoluble in hydrochloric or nitric acids. The test would naturally be valueless had any sulphate, such as that of zinc, been given by the mouth.

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THE END

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